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The call by which the students of medicine from Tuzla (Bosnia and Herzegovina), at the suggestion of their professor of pharmacology Rajko Igić, started anti- smoking campaign in 1981. Later this day, January 31, first in Serbia, and then in Bosnia and Herzegovina, was declared for the National Day against Smoking.

Poziv kojim su studenti medicine iz Tuzle (Bosna i Hercegovina), na predlog svog profesora farmakologije Rajka Igića, započeli kampanju protiv pušenja 1981. godine. Kasnije je ovaj dan, 31. januar, najpre u Srbiji, a zatim i u Bosni i Hercegovini, proglašen za Nacionalni dan borbe protiv pušenja.

E D I T O R I A L

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Novelity in the new 2018

Novo u novoj 2018. godini

Silva Dobrić

University of Defence, Institute for Scientific Information, Belgrade, Serbia

The main novelity regarding the Vojnosanitetski Pregled (VSP) in the new 2018 is the change of its publisher. Instead of the Military Health Department of the Ministry of Defence of the Republic of Serbia which, since of the Journal founding in 1944 was its publisher, will be the University of Defence in future. Namely, on December 1, 2017, the Institute for Scientific Information of the Military Medical Academy in Belgrade, in which the Journal's Editorial Office was situated since 1961, became a member of the University of Defence, which took over all the duties related to the Journal. The University of Defence will continue to publish, along with the Vojnosanitetski Pregled, additional three scientific military journals, and thus encompass the role of high education and scientific institution. This change will not affect the editorial policy of the VSP. Moreover, we expect to help solve some technical and financial issues in publishing the Journal, which will further strengthen its position as the leading medical journal in Serbia with constant increase of impact factor over the last several years. That is why it is not surprising that for years the number of manuscripts, which almost each day reach the Journal`s Editorial Office, is growing.

In the year 2017, to December 15 inclusive, a total of 343 manuscripts were received, similarly to the number of the previous years, which ranged from 300 to 400. Of this number, aproximately 30% of manuscripts has been rejected, while the remaining have received positive reviews or are still in the process of reviewing.

As in previous years, the largest number of manuscripts received belongs to the category of Original Article (67.64%) and Case Report (21.28%). Next are those from the categories General Review (4.96%) and Current Topic (2.04%), while other categories of manuscripts (Short Communication, Letter to the Editor, History of Medicine, Practical Advice for the Physicinas, etc.) are represented by less than 1%.

When analyzing the affiliation of the authors of the manuscripts received, it is evident that, as earlier, the largest number of them was sent by domestic authors from the so-called civil health and academic institutions (87.17%), about 7% of manuscripts were submitted by authors from abroad, while approximately 6% from authors from Serbian military institutions.

The analysis of the articles published in 2017 has showed that their structure is largely the same as in previous years. As to now, most articles published have been from the category Original Article, followed by Case Report, while a significantly smaller number of articles have been from other categories (Table 1). Again, most articles published were by domestic authors from civil institutions (99), followed by joint articles of authors from civil and military institutions (31). At the third place, there were articles by authors from military health institutions (24), while number of articles published by authors from abroad, alone or in combination with domestic authors, was 11 and 14, respectively.

Table 1
Categories and the number of articles published in the Vojnosanitetski Pregled in 2017

Category of articles	Articles	
	n	%
Editorial	3	1.7
Original Article	91	50.9
General Review	8	4.5
Current Topic	4	2.2
Practical Advice for Physicians	2	1.1
Case Report	48	26.9
Short Communication	14	7.8
In Focus	1	0.5
History of Medicine	5	2.8
Letter to the Editor	2	1.1
Meeting Report	1	0.5
Total	179	100

Over the last year, efforts were made to select only those papers with the topics which follow world science trends. This will continue to be one of the priority tasks of the Publisher and the Editorial Board of the Journal in the future. Great help in this we expect from our reviewers to whom, on this occasion, I warmly thank on behalf the members of the Editoral Board and Editorial Office of the Journal. The names of those who helped us achieve our common goal in 2017 are listed in Table 2.

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Table 2

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Oral health-related quality of life among Belgrade adolescents

Kvalitet života u funkciji oralnog zdravlja beogradskih adolescenata

Milica Gajić, Maja Lalić, Katarina Kalevski, Marjan Marjanović

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Abstract

Background/Aim. Adolescents are vulnerable group in term of acquisition of oral health-related knowledge, habits and attitudes. That is why the aim of this study was to investigate the associations between dental status, dental anxiety and oral health-related behavior and oral health-related quality of life as captured by Oral Impacts on Daily Performances (OIDP) index. **Methods.** This cross-sectional survey included representative sample of 404 adolescents (15 years old), randomly recruited from high schools in Belgrade, Serbia. The adolescents were interviewed using Serbian versions of eight-item OIDP index, Hiroshima University Dental Behavior Inventory (HU-DBI) and modified Corah's Dental Anxiety Scale (MDAS). Three previously trained and calibrated dentists examined the subjects in the classrooms to determine the oral health status of adolescents [the Decayed, missing, filled teeth (DMFT) index and visual signs of gingivitis]. **Results.** At least one oral impact was reported in 49.50% of adolescents. Most frequently, oral health problems affected eating (26.73%), tooth cleaning (27.47%) and sleep and relaxation (16.83%). In comparison with adolescents without oral impacts, the adolescents with at least one oral impact reported, had higher DMFT score, more often reported problems with bleeding gums, usage of hard toothbrush, worries about the color of their teeth and seeing the dentist because of the symptoms. Logistic regression showed that dental anxiety (MDAS score), dental behavior (HU-DBI score) and worrying about the color of the teeth significantly affected OIDP score. **Conclusion.** Oral health-related quality of life among adolescents was affected by their behavior and dental anxiety levels. Implementing public health policies that target adolescents with poor oral health or bad habits might be helpful in improving their oral health-related quality of life.

Key words:
adolescent; oral health; quality of life; surveys and questionnaires; serbia.

Apstrakt

Uvod/Cilj. Adolescenti predstavljaju osetljiviji deo populacije u smislu sticanja znanja, navika i odnosa prema oralnom zdravlju. Stoga, cilj istraživanja je bio da se utvrdi povezanost stanja oralnog zdravlja, straha od stomatologa i ponašanja u vezi sa oralnim zdravljem i njegovog uticaja na kvalitet života. Merenje je izvršeno pomoću upitnika „Uticaj oralnog zdravlja na svakodnevne aktivnosti" (OIDP). **Metode.** Studijom preseka bila su obuhvaćena 404 adolescenta uzrasta od 15 godina, odabrana metodom slučajnog uzorka iz srednjih škola sa područja Beograda. Adolescenti su anketirani pomoću srpskih verzija upitnika OIDP, upitnika Univerziteta Hirošima za procenu ponašanja u vezi sa oralnim zdravljem (HU-DBI) i modifikovane Korahove skale dentalne anksioznosti (MDAS). Tri prethodno obučena i standardizovana istraživača pregledala su ispitanike u učionicama kako bi utvrdili stanje njihovog oralnog zdravlja [indeks karijes, ekstrakcija, plomba (KEP) i prisustvo gingivitisa]. **Rezultati.** Najmanje jedan negativan uticaj na oralno zdravlje zabeležen je kod 49,50% adolescenata. Problemi u vezi sa oralnim zdravljem najčešće su ometali ishranu (26,73% adolescenata), pranje zuba (27,47%), san i odmor (16,83%). Kod adolescenata sa barem jednim negativnim uticajem oralnog zdravlja na svakodnevne aktivnosti, zabeležen je viši KEP indeks, češće krvarenje desni, upotreba tvrde četkice za zube, zabrinutost zbog boje zuba i odlazak kod stomatologa zbog simptoma, u poređenju sa ispitanicima kod kojih oralno zdravlje nije uticalo na kvalitet života. Logističkom regresijom utvrđeno je da dentalna anksioznost (MDAS skor), ponašanje u vezi sa oralnim zdravljem (HU-DBI skor) i zabrinutost zbog boje zuba značajno utiču na kvalitet života meren pomoću upitnika OIDP. **Zaključak.** Ponašanje adolescenata kada je u pitanju oralno zdravlje i strah od stomatologa utiču na njihov kvalitet života. Uvođenje javnozdravstvenih strategija za zaštitu adolescenata sa lošim oralnim zdravljem i lošim navikama mogao bi da unapredi njihov kvalitet života.

Ključne reči:
adolescenti; usta, zdravlje; kvalitet života; ankete i upitnici; srbija.

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Introduction

Adolescents are vulnerable group in terms of acquisition of oral health-related knowledge, habits and attitudes. Due to their dynamic physical and psychoemotional development, the pre-established habits are being changed and new patterns of behavior and value systems are being formed under the influence of their immediate environment, peers and informal groups, which might increase the risk of impairing one's health. Smoking, inadequate nutrition, "forgetting" about the oral hygiene, and a lack of understanding of the risks arising from existing forms of inadequate health behavior, may adversely affect the oral health of adolescents [1]. Risk behavior not only affect clinical aspects of oral health, but may also have a negative impact on adolescents' oral health- related quality of life. To measure oral health-related behavior, Kawamura [2] developed Hiroshima University Dental Behavior Inventory (HU-DBI) which contains twenty questions mostly related to oral hygiene behavior. Studies that compared the oral health- related attitudes and behaviors among dental students around the world, using HU-DBI questionnaire translated into several languages, revealed significant differences among students from different countries and cultural groups [3, 4]. This indicates that HU-DBI index can be used to assess dental behavior worldwide.

The modern concept of oral health care for adolescents implies that the focus of attention is transferred from clinical parameters onto broader health determinants - psychological, social and physiological. Impact of impaired oral health on everyday life is subtle and pervasive, influencing eating, sleep, work and social roles. Therefore, comprehensive evaluation of oral health should not be based solely on clinical data but connected with clinical findings. In order to assess the impact of oral health conditions on quality of life, different scales have been developed. Most of them are designed and tested on adult population. Among the scales that measure the impact of oral health on daily activities of adolescents for their reliability and validity in different cultural environments the one that stands out is "Oral Impacts on Daily Performances" (OIDP) [5]. Serbian version of OIDP was introduced in 2012 and applied to geriatric population [6].

The aim of this study was to examine how dental anxiety, oral health-related behavior and clinical parameters of adolescents oral health affect their oral health-related quality of life as captured by OIDP index.

Methods

This cross-sectional study included 404 first grade secondary school students (aged 15 years). Subjects were randomly recruited from the list provided from ten previously randomly selected schools from different municipalities in the city of Belgrade.

Data regarding oral health behavior were collected using a Serbian version of the English HU-DBI [2]. Questions have two possible forms of answers (agree/disagree). Quantitative assessment of the attitudes and behaviors related to oral health can be determined on the basis of the total number of adequate responses with a maximum score of 12. Higher score indicates more appropriate oral health-related attitudes and behavior [7]. In calculating the HU-DBI score, 1 point was awarded for each "agree" answer to questions 4, 9, 11, 12, 16 and 19, and for each "disagree" answer to questions 2, 6, 8, 10, 14 and 15. Three additional questions regarding oral hygiene and smoking habits were included in the final Serbian version of HU-DBI questionnaire, with no impact on HU-DBI score.

The estimation of the quality of life in relation to oral health of adolescents was measured using OIDP questionnaire. The OIDP index refers to oral impacts the subjects experienced due to their mouth and teeth problems, during the previous 6 months period, in relation to: 1) eating, 2) speaking and pronouncing clearly, 3) cleaning teeth, 4) sleeping and relaxing, 5) smiling without embarrassment, 6) maintaining emotional state, 7) enjoying contact with other people and 8) carrying out major school work. The scale used was in the range: (0) "never" or "less than once a month", (1) "once or twice a month", (2) "once or twice a week" (3) "3–4 times a week", (4) "every or nearly every day". We used the shortened version of the OIDP questionnaire with unweighted frequency scores. [6] Total score was calculated by adding the 8 OIDP items, with the possible scope ranges from 0 to 32. For analysis, dummy variables were constructed yielding the categories 0 = "never affected" (including the original category 0) and 1 = "affected less than once a month or more often" (including the original categories 1–4).

Dental anxiety levels in adolescents were measured using a modified version of Corah's Dental Anxiety Scale (MDAS) [8]. This scale estimates respondents' feelings the day before going to the dentist, during his/her stay in the waiting room, in the chair before the intervention starts, at the beginning of the intervention and while receiving anesthesia. Answers were ranked on a scale from 0 to 4, with 0 – denoting not frightened at all; 1 – a little frightened; 2 – moderately frightened; 3 – quite frightened; 4 – extremely frightened.

Clinical dental examination was undertaken by three previously trained and calibrated dentists in school classrooms, under natural light. The number of healthy, decayed, filled and missing teeth (DMFT) index was recorded, as well as the presence of the visual signs of gum inflammation (redness and gingival enlargement).

The SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) program was used to analyze the data. Simple frequency tables and descriptive statistics (means and standard deviations) were processed and analyzed by χ^2 and Fisher's exact tests. Differences in DMFT score and its components in relation to agree/disagree responses of HU-DBI questionnaire were assessed using independent samples *t*-test.

Pearsons' correlation coefficient was calculated for OIDP items. The influence of clinical parameters for assessing oral health, oral health-related behavior and dental anxiety on the quality of oral health was evaluated using logistic regression.

The significance level established for all analyses was $p < 0.05$.

Results

Clinical examination

All healthy teeth (DMFT = 0) were found in only 35 (8.66%) patients. Less than a third of respondents (119 adolescents, 29.46%) had no untreated teeth at the time of examination decayed or carious teeth (DT) = 0]. Average number of [decayed teeth was DT = 2.65 ± 0.14 (Table 1). Average number of filled teeth (FT), which represents treated dental pathology, was mean ± SD = 2.71 ± 0.14, denoting a rather low dental treatment rate. The presence of gingival inflammation (redness, swelling and/or bleeding from marginal gingiva) was observed in 74.9 % of adolescents.

and 22 (Table 3). Agreement with the statement "I go to see the dentist at least ones a year" was related with significantly lower DMFT ($p < 0.01$) and FT ($p < 0.05$). Agreement with the statement "My gums bleed when I brush my teeth" was related to higher DMFT score, DT and MT components ($p < 0.05$). A significant correlation was observed between negative attitude "I think that I cannot help having false teeth when I am old" and high MT component ($p < 0.05$). Subjects who agreed with the statement "I think my teeth are getting worse despite my daily brushing" had higher DMFT score and higher DT component ($p < 0.01$). Subjects who received feedback from their dentist regarding their brushing efficacy had lower DMFT score, DT and MT components ($p < 0.01$).

Table 1

Hiroshima University-Dental Behavioural Inventory (HU-DBI) score and oral health status of adolescents [composition of decayed, missing, filled teeth (DMFT) index] according to the gender

Parameter	Males	Females	*p**
	x̄ ± SD	x̄ ± SD	
HU-DBI score	6.22 ±1.44	6.28 ±1.450	0.674
Healthy teeth, n	23.24 ± 3.396	21.88 ± 4.325	0.003
Decayed teeth, n	2.77 ± 3.005	2.61 ± 2.685	0.594
Missing teeth, n	0.28 ± 0.679	0.58 ± 1.033	0.005
Filled teeth, n	1.93 ± 2.067	2.99 ± 3.077	0.001
DMFT	4.95 ± 6.16	3.357 ± 4.214	0.003

x̄ – mean value; SD – standard deviation; * *p* value for Fisher's χ^2 – test.
***p* < 0.05 was considered statistically significant.**

Oral health- related behavior

Adolescents expressed moderately acceptable oral health-related behavior measured by HU-DBI questionnaire. HU- DBI score ranged from 2 to 11 with average value of 6.27 ± 0.27. The female subjects had a higher score (6.28 ± 1.45) compared with male subjects (6.22 ± 1.45; $p < 0.05$). HU-DBI questionnaire items and percentage of agree/disagree responses are presented in Table 2. Dental visits at least once a year were reported by 67.3% adolescents, more often by girls ($p < 0.05$). Bleeding gums were reported by 18.4% of participants; 65.1% answered that it was impossible to prevent gum disease with only toothbrushing. Majority of subjects (70.7%) reported that they had been professionally taught how to brush their teeth, girls more often than boys ($p < 0.05$). Toothpaste was considered necessary for brushing in 91.8% of subjects, more often among girls ($p < 0.05$). Almost half of the subjects used toothbrush with hard bristles and brushed with hard strokes, girls more often than boys ($p < 0.05$). Postponing of dental visits until toothache was reported by 47.1% of adolescents. Majority of subjects reported brushing twice a day or more often (86.2%), girls more often than boys ($p < 0.001$), but only 13.4% reported regular flossing and 30.3% daily use of mouth rinses. Nearly one quarter of adolescents (23.3%) reported smoking cigarettes every day.

Significant differences between DMFT, DT, missed teeth (MT) or FT values in relation to agree/disagree HU-DBI responses were found in Items 1, 2, 5, 6, 13, 14, 16, 18, 19

Those who were satisfied with the appearance of their teeth had significantly lower DMFT score, DT, MT and FT components ($p < 0.01$).

Dental anxiety

Adolescents expressed moderate dental anxiety levels. Dental anxiety score ranged from 5 to 25, with the mean of 12.16 ± 5.47. Girls were signifficantly more anxious compared with boys (12.86 ± 5.54 vs. 10.36 ± 4.8, respectively; $p = 0.001$).

Oral health-related quality of life

At least one oral impact was reported in 49.50% of adolescents. The frequency of oral impacts was greater in females (53.08%) than males (40.54%), $\chi^2 = 5.06$; $p = 0.024$. Oral health most frequently affected eating and enjoying food (26.73%), tooth cleaning (27.47%), and sleep and relaxation (16.83%), while least severe impacts affected speaking and pronouncing words (6.19%) and social life of adolescents (6.93%).

Table 4 shows correlation matrix for OIDP frequency scores (1–8). The inter-item correlation coefficients among the eight OIDP items ranged from 0.05 (between eating and emotional status) to 0.57 (between showing teeth and carrying out work). There were no negative correlation coefficients, indicating the homogeneity among the items.

Table 2

Percentage of agree and disagree Hiroshima University-Dental Behavioural Inventory (HU-DBI) items according to the gender

HU-DBI Item	Gender, n (%)		Total	*p*
	M	F		
1. I go to see the dentist at least ones a year				
agree	84 (21.0)	185 (46.3)	269 (67.3%)	0.032
disagree	27 (6.8)	104 (26.0)	131 (32.8%)	
2. My gums bleed when I brush my teeth				
agree	24 (6.0)	50 (12.4)	74 (18.4)	0.316
disagree	87 (21.6)	241 (60.0)	328 (81.6)	
3. I am worried about the color of my teeth				
agree	50 (12.5)	106 (26.6)	156 (39.1)	0.135
disagree	60 (15.0)	183 (45.9)	243 (60.9)	
4. I've noticed some white sticky deposits on my teeth				
agree	20 (5.0)	42 (10.5)	62 (15.5)	0.440
disagree	91 (22.7)	248 (61.8)	339 (84.5)	
5. I think that I cannot help having false teeth when I am old				
agree	30 (7.5)	81 (20.3)	111 (27.8)	0.901
disagree	81 (20.3)	208 (52.0)	289 (72.3)	
6. I think my teeth are getting worse despite my daily brushing				
agree	21 (5.2)	35 (8.7)	56 (14.0)	0.106
disagree	90 (22.4)	255 (63.6)	345 (86.0)	
7. I brush each of my teeth carefully				
agree	56 (13.9)	146 (36.2)	202 (50.1)	1.000
disagree	55 (13.6)	146 (36.2)	201 (49.9)	
8. I have never been professionally taught how to brush				
agree	42 (10.4)	76 (18.9)	118 (29.3)	0.027
disagree	69 (17.1)	216 (53.6)	285 (70.7)	
9. I think I can clean my teeth without using toothpaste				
agree	15 (3.7)	18 (4.5)	33 (8.2)	0.024
disagree	96 (23.8)	274 (68.0)	370 (91.8)	
10. I often check my teeth in a mirror after brushing	101 (25.1)	279 (69.2)	380 (94.3)	0.093
	10 (2.5)	13 (3.2)	23 (5.7)	
11. I worry about having bad breath.	91 (22.9)	236 (59.4)	327 (82.4)	0.907
	19 (4.8)	51 (12.8)	70 (17.6)	
12. It is impossible to prevent gum disease with tooth brushing alone	63 (16.1)	192 (49.0)	255 (65.1)	0.281
	41 (10.5)	96 (24.5)	137 (34.9)	
13. I put off going to the dentist until I have a toothache	53 (13.2)	136 (33.9)	189 (47.1)	0.823
	57 (14.2)	155 (38.7)	212 (52.9)	
14. I have used a dye to see how clean my teeth are	8 (2.0)	22 (5.5)	30 (7.5)	0.927
	101 (25.4)	267 (67.1)	368 (92.5)	
15. I use a toothbrush with hard bristles	42 (10.6)	142 (35.9)	184 (46.5)	0.034
	69 (17.4)	143 (36.1)	212 (53.5)	
16. I don't feel I've brushed well unless I brush with strong strokes	68 (16.9)	143 (35.5)	211 (52.4)	0.034
	43 (10.7)	149 (37.0)	192 (47.6)	
17. I feel I sometimes take too much time to brush my teeth	65 (16.1)	157 (39.0)	222 (55.1)	0.433
	46 (11.4)	135 (33.5)	181 (44.9)	
18. I have had my dentist tell me that I brush very well	66 (16.8)	195 (49.5)	261 (66.2)	0.281
	41 (10.4)	92 (23.4)	133 (33.8)	
19. I am satisfied with the appearance of my teeth	62 (15.5)	156 (38.9)	218 (54.4)	0.738
	49 (12.2)	134 (33.4)	183 (45.6)	
20. I brush my teeth twice daily or more	78 (19.4)	268 (66.7)	346 (86.1)	0.0001
	33 (8.2)	23 (5.7)	56 (13.9)	
21. I use dental floss every day	11 (2.7)	43 (10.7)	54 (13.4)	0.252
	100 (24.9)	248 (61.7)	348 (86.6)	
22. I use mouthwash on regular basis	29 (7.3)	92 (23.0)	121 (30.3)	0.331
	81 (20.3)	198 (49.5)	279 (69.8)	
23. I smoke cigarettes every day	27 (6.7)	67 (16.6)	94 (23.3)	0.793
	84 (20.8)	225 (55.8)	309 (76.7)	

***p* value for Fisher's χ^2 test ($p < 0.05$ was considered statistically significant); M – male; F – female.**

Table 3

Mean decayed, missing, filled teeth (DMFT) scores and their decayed teeth (DT), missing teeth (MT) and filled teeth (FT) components in relation to the agreement/disagreement to the Hiroshima University Dental Behavioural Inventory (HU-DBI) items

	HU-DBI Item	DMFT	DT	MT	FT
1	agree/disagree	5.44**/6.69**	2.51/2.93	0.50/0.51	2.46*/3.26*
2	agree/disagree	6.80*/5.60*	3.32*/2.49*	0.81**/0.43**	2.75/2.69
3	agree/disagree	6.19/5.60	2.96/2.45	0.54/0.48	2.75/2.67
4	agree/disagree	6.44/5.71	3.22/2.54	0.58/0.48	2.69/2.71
5	agree/disagree	6.08/5.74	2.73/2.63	0.71*/0.42*	2.70/2.70
6	agree/disagree	7.18**/5.63**	3.93**/2.46**	0.52/0.50	2.78/2.69
7	agree/disagree	5.87/5.81	2.61/2.69	0.54/0.46	2.77/2.66
8	agree/disagree	5.59/5.94	2.67/2.64	0.42/0.53	2.53/2.79
9	agree/disagree	5.36/5.88	2.30/2.68	0.61/0.49	2.45/2.73
10	agree/disagree	5.85/5.61	2.65/2.65	0.49/0.61	2.73/2.45
11	agree/disagree	5.98/5.29	2.73/2.34	0.52/0.44	2.77/2.50
12	agree/disagree	5.89/5.77	2.75/2.38	0.53/0.45	2.63/2.96
13	agree/disagree	5.67/5.96	2.86/2.48	0.49/0.51	2.34*/2.99*
14	agree/disagree	7.23*/5.72*	3.40/2.56	0.63/0.49	3.31/2.68
15	agree/disagree	6.17/5.60	2.72/2.58	0.59/0.42	2.90/2.62
16	agree/disagree	6.15/5.49	2.99*/2.28*	0.62**/0.36**	2.57/2.87
17	agree/disagree	5.91/5.74	2.80/2.47	0.51/0.48	2.63/2.81
18	agree/disagree	5.42**/6.80**	2.43**/3.20**	0.40**/0.71**	2.62/2.91
19	agree/disagree	4.89***/6.96***	2.07***/3.35***	0.41*/0.61*	2.44*/3.01*
20	agree/disagree	5.92/5.39	2.68/2.54	0.50/0.50	2.75/2.44
21	agree/disagree	6.09/5.76	2.83/2.61	0.46/0.49	2.80/2.68
22	agree/disagree	6.61*/5.51*	3.02/2.49	0.57/0.47	3.03/2.59
23	agree/disagree	5.84/5.84	2.73/2.63	0.37/0.54	2.80/2.68

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.**

Note: for HU-DBI items see Table 2.

Table 4

Correlation matrix for Oral Impacts on Daily Performances (OIDP) frequency scores (1–8)

OIDP	Eating	Speaking	Cleaning teeth	Sleeping/ relaxing	Showing teeth	Emotional status	Carrying out work	Enjoying social contact
Eating								
r	1							
p								
Speaking								
r	0.267**	1						
p	< 0.001							
Cleaning teeth								
r	0.442**	0.300**	1					
p	< 0.001	< 0.001						
Sleeping/ relaxing								
r	0.440**	0.399**	0.418**	1				
p	< 0.001	< 0.001	< 0.001					
Showing teeth								
r	0.147**	0.288**	0.231**	0.169**	1			
p	0.003	< 0.001	< 0.001	0.001				
Emotional status								
r	0.048	0.290**	0.167**	0.337**	0.160**	1		
p	0.334	< 0.001	0.001	< 0.001	0.001			
Carrying out work								
r	0.222**	0.277**	0.153**	0.283**	0.574**	0.283**	1	
p	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001		
Enjoying social contact								
r	0.100*	0.144**	0.161**	0.354**	0.207**	0.495**	0.308**	1
p	0.044	0.004	0.001	< 0.001	< 0.001	< 0.001	< 0.001	

***r* – Pearson's correlation coefficient.**

After OIDP score was dichotomized into 2 categories: 1) no oral impacts (OIDP score = 0) and 2) presence of oral impacts (OIDP score > 0), oral health behavior of subject who reported one or more oral impacts was compared with subjects with no oral impacts. Subjects with one or more oral impacts more frequently reported: gums bleeding during tooth brushing ($\chi^2 = 10.28$; $p = 0.001$); worries about the color of their teeth ($\chi^2 = 9.18$; $p = 0.002$); presence of white sticky deposits on their teeth ($\chi^2 = 4.78$; $p = 0.029$); belief that having false teeth was inevitable in older age ($\chi^2 = 13.07$; $p = 0.0001$); delaying to see the dentists until they had toothache ($\chi^2 = 21.95$; $p = 0.0001$); brushing their teeth by using strong strokes ($\chi^2 = 19.30$; $p = 0.0001$); less satisfaction with the appearance of their teeth ($\chi^2 = 28.06$; $p = 0.0001$).

No significant differences regarding dental visits pattern, brushing frequency, use of dental floss and mouth washes and smoking habit were obtained in subjects who reported one or more oral impacts compared to those with no impacts.

Adolescents who had untreated decayed teeth had greater mean OIDP score compared to those with fully treated teeth (F = 6.39; $p = 0.42$). Those with positive history of tooth extraction had greater oral impacts compared to those with no teeth extracted (F = 1.94; $p = 0.25$).

Oral health-related quality of life was better among adolescents who self-rated their oral health as good (OIDP = 1.47 ± 2.80), compared with those who percieved their oral health as moderately good (OIDP = 2.44 ± 3.53), or poor (OIDP = 4.55 ± 5.99; F = 10.81; $p = 0.001$).

Correlation among HU- DBI, OIDP and MDAS score, DMFT score, DMFT components and presence of gingival inflammation were represented in Table 5. Logistic regression showed that MDAS score, HU-DBI index and worrying about the color of the teeth predominantly affected OIDP score.

Table 5

Correlation of Hiroshima University Dental Behavioural Inventory (HU-DBI) score, Oral Impacts on Daily Performances (OIDP) and Modified Dental Anxiety Scale (MDAS) score, decayed, missing, filled teeth (DMFT) score and its components, and presence of gingival inflammation

		DBI	Gingivitis	OIDP	MDAS	DMFT	DT	MT	FT
DBI									
	r	1							
	p								
	n	404							
Gingivitis									
	r	-0.0248	1						
	p	0.6208							
	n	400	400						
OIDP									
	r	-0.1526	-0.0586	1					
	p	0.0021	0.2420						
	n	404	400	404					
MDAS									
	r	-0.1789	-0.0581	0.1045	1				
	p	0.0003	0.2463	0.0358					
	n	404	400	404	404				
DMFT									
	r	-0.0166	-0.3256	0.0629	0.0451	1			
	p	0.7401	< 0.0001	0.2069	0.3655				
	n	404	400	404	404	404			
DT									
	r	-0.0799	-0.5272	0.1339	0.1215	0.6028	1		
	p	0.1088	< 0.0001	0.0070	0.0146	< 0.0001			
	n	404	400	404	404	401	404		
MT									
	r	-0.0384	-0.0508	0.0827	0.0512	0.4430	0.0600	1	
	p	0.4430	0.3125	0.0980	0.3065	< 0.0001	0.2304		
	n	401	397	401	401	401	401	401	
FT									
	r	0.0639	0.0600	-0.0619	-0.0702	0.6736	-0.1372	0.2351	1
	p	0.2017	0.2328	0.2163	0.1604	< 0.0001	0.0059	< 0.0001	
	n	401	397	401	401	401	401	399	401

DBI – Dental Behavioural Inventory; DT – decayed teeth; MT – missing teeth; FT – filled teeth; *r* – Pearson's Correlation coefficient; *p* – *p* value for Fisher's χ^2 test ; n – the number of respondents/students.

Discussion

The participants of this study, were randomly selected from the population of secondary schools in Belgrade. Thus, they might have captured the variety of characteristics of 15-year-old adolescents attending secondary schools in the Serbian capital.

In our study adolescents reported acceptable oral hygiene behavior, with 86% of adolescents brushing teeth

twice a day, but high prevalence of gingivitis indicated inadequate brushing. In a study of brushing behavior in children from 32 European countries and North America, authors reported that percentage of children who brush their teeth more than once a day ranged from 16% to 80% among boys and from 26% to 89% among girls, with tendancy to increase with child age in some countries, and decrease in other countries [9].

Our study revealed that nearly half of subjects postponed dental visit until toothache appear, and 67% of adolescents reported at least one dental visit within past year. Sympthom related dental visits prevailed over regular checkups. In The Serbian Population Health Survey conducted by the Serbian Ministry of Health in 2006 [10], decrease in dental visits once a year, from 36.8% to 30.7% from 2000 to 2006, but also the increase in the number of regular dental checkups, were reported. However, compared to the year of 2000, the percentage of children and youth who visited a dentist in the previous year, increased from 58.9% to 63.7%.

In the Central and Eastern European countries, increased prevalence of dental caries in school children and adolescents is associated with inconsistent implementation of preventive measures and lack of organized health promotion activities [11]. Also, high prevalence of caries in developing countries may be partly explained by the fact that the health system of these countries is still in transition [12]. Unfortunately, since health promotion activities in Serbia are not systematicaly and consistently implemented and the health care system is oriented toward treatment rather than prevention of oral diseases, high DMFT score in 15-year- old adolescents is not surprising.

Adolescents oral health status determined in this study, in terms of realized risk of developing caries and periodontal disease, is characterized by high prevalence of dental disease (with 91% of adolescents with DMFT > 0), with 45% of untreated dental decay (DT/DMFT). In a total sample, mean DMFT score was 5.84. Some other studies reported that average DMFT score in group of 15 years old subjects was 1.8 in Germany [13], 3.19 in Greece [14], 4.3 in Slovenia [15], and 6.6 in Bosnia [16].

Better oral health-related behaviors were associated with better dental status. OIDP score was affected by dental behavior, dental anxiety levels and clinical parameters of oral health. Consistent with the results reported in OIDP surveys [17, 18], difficulty with eating and enjoying food and cleaning teeth were the impacts most frequently reported. Untreated dental caries and history of tooth extraction were related to higher oral impact score indicating good discriminant validity of Serbian version of OIDP scale. Higher OIDP score was related with poor self-perceived oral health. Adolescents mostly rated their oral health as good, similarly to previous findings [19]. Östberg et al. [20] reported that adolescents usually gave insufficient priority to oral health, e.g. tooth cleaning, fluoride supplements and diet habits, being unaware of their own respectively regarding oral health.

Our findings might be useful in setting oral health goals and determining treatment needs in population of Serbian adolescents, as suggested by other authors [21]. Organization of oral health care should be planned on the basis of dental care needs. The information most commonly used in the organization of oral health care, is population dental caries experience and prevalence of clinically detectable oral health problems. However, service planning should include wider, psychosocial determinants of oral health [22], since they could affect people's everyday life in a significant manner.

Conclusion

Oral health-related quality of life in adolescents in Belgrade was affected by their behavior, dental anxiety and oral health state. Public health policies that address adolescents with poor oral health might be helpful in improving both clinical and psychosocial determinants of oral health as well as their oral health-related quality of life.

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The prevalence of coronary artery anomalies in adults: studied with computed tomography coronary angiography

Prevalenca koronarnih anomalija kod odraslih: ispitivanje kompjuterizovanom tomografskom koronarnom angiografijom

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Abstract

Background/Aim. Coronary artery anomalies are an uncommon but important cause of chest pain, and in some cases of hemodynamically significant abnormalities, sudden cardiac death. The aim of the research was to establish the prevalence of the coronary arteries anomalies in our population. **Methods.** The study group included 1,562 patients (810 men, 752 women, average age 64.3 ± 12.0 years; range 32–80 years) who were scheduled for 64-slice computed tomography (MSCT), which enables detailed visualization of coronary arteries and heart anatomy. All examinations were made due to suspicion (atypical chest pain, angina equivalent symptoms or multiple risk factors for cardiovascular disease) or assumption of progression of coronary artery disease. **Results.** From January 2010 till December 2014 a total number of 1,562 patients were sent for evaluation of coronary arteries. The coronary anomalies were found in 45 (2.88%) patients. The most frequent coronary anomaly seen in our population group was absence of left main trunk with the separate origin of the left anterior descending artery (LAD) and left circumflex artery (LCx) originating from a left coronary sinus (LCS). This was found in 12 patients (an incidence of 0.77% or 26.7% of all coronary anomalies). Anomalous location of coronary ostium outside normal aortic sinuses in our study was present as right coronary artery (RCA) that arises from left anterior sinus in 5 (0.32%) patients and left coronary artery from non-coronary sinus in two (0.13%) patients. **Conclusion.** Knowledge of anomalies of the coronary arteries and their recognition on the multislice computed tomography is of great importance for the further planning of a possible therapeutic treatment. Coronary anomalies that are considered insignificant will require no further therapeutic treatment. But the detection of malignant coronary anomalies will certainly save many lives.

Key words:
coronary vessels; adult; abnormalities; coronary angiography; prevalence.

Apstrakt

Uvod/Cilj. Anomalije koronarnih arterija su redak, ali važan uzrok bola u grudnom košu tako da, u nekim slučajevima, hemodinamski značajne abnormalnosti mogu da izazovu iznenadnu srčanu smrt. Cilj istraživanja bio je da se utvrdi prevalencija anomalija koronarnih arterija u našoj populaciji. **Metode.** Istraživanje je uključilo 1 562 bolesnika (810 muškaraca, 752 žene, prosečne starosti 64,3 ± 12,0 godina, raspon 32–80 godine) koji su pregledani na 64-slajsnoj kompjuterizovanoj tomografiji (MSCT). Pregled na ovom apartu omogućuje detaljnu vizualizaciju koronarnih arterija i srčane anatomije. MSCT pregledi su obavljeni na bolesnicima zbog sumnje na postojanje koronarne bolesti (atipični bol u grudima, angina pektoris ili više faktora rizika od kardiovaskularnih bolesti), kao i moguće progresije bolesti koronarnih arterija. **Rezultati.** Od januara 2010. do decembra 2014. godine pregledano je ukupno 1 562 bolesnika za procenu starenja koronarnih arterija. Incidenca srčanih anomalija je bila zastupljena kod 45 bolesnika (2,88%). Koronarna anomalija koja je bila najzastupljenija u našoj studiji je bilo odsustvo glavnog stabla leve koronarne arterije sa odvojenim ishodištima leve descedentne arterije (LAD) i leve cirkumfleksne arterije (LCX) iz levog koronarnog sinusa (LCS). Pronađena je kod 12 bolesnika (učestalost od 0,77%, odnosno 26,7% svih srčanih anomalija). U našoj studiji anomalije ishodišta koronarnih arterija van normalnog koronarnog sinusa manifestovale su se kao anomalno ishodište desne koronarne arterije (RCA) iz levog koronarnog sinusa kod 5 (0,32%) bolesnika i leve koronarne arterije iz nekoronarnog sinusa kod dva (0,13%) bolesnika. **Zaključak.** Poznavanje anomalija koronarnih arterija i njihova vizualizacija na višerednoj kompjuterizovanoj tomografiji od velike je važnosti za dalje planiranje mogućeg terapijskog tretmana. Koronarne anomalije koje se smatraju beznačajnim neće zahtevati dalji terapijski tretman. Ali, otkrivanje hemodinamski značajnih koronarnih anomalija sigurno će spasiti mnoge živote.

Ključne reči:
koronarni krvni sudovi; odrasle osobe; anomalije; angiografija koronarnih arterija; prevalenca.

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Introduction

Coronary artery anomalies are defined as an abnormality of the origin, direction, and coronary artery bifurcations. This definition of anomalies of the coronary arteries is incomplete, as we still do not have any recommendations for the diagnosis and treatment of these anomalies. The biggest obstacle to solving this problem is a great variety of clinical presentations (from completely silent to those that can lead to sudden cardiac death), as well as an incomplete understanding of the pathophysiology of these disorders.

The most widely accepted attitude about these anomalies was given by Angelini [1–3] that they "can be considered a normal variation of each coronary artery that has a frequency of > 1% in the general population".

It is enough to ask ourselves whether anatomical variation that does not cause any functional impairment, which is seen very rarely, such as, for example, the separate origin of the circumflex branch of the left coronary artery, deserves to be classified as a coronary anomaly or not.

Methods

The study group included 1,562 patients (810 men, 752 women, average age 64.3 ± 12.0 years; range 32–80 years) which were scheduled for multislice computed tomography (MSCT) which enables detailed visualization of coronary arteries and heart anatomy. They were referred to the Department of Radiology of the Clinical Center Niš, Serbia. Exclusion criteria were patients with previous allergic reaction to iodinated contrast, pregnant women, heart rate irregularity and renal insufficiency (creatinine ≥ 1.5 mg/dL).

Examinations were performed using a Multi-Slice Computed Tomography Toshiba Aquilion 64 system (Toshiba Medical Systems, Japan), with a rotation time of 0.33 seconds and a collimation of 64 × 0.5 mm. The tube current was 120 kV, at 300 mA. Field of view (FOV) was 140–180 mm.

Nonionic contrast material was applied in the cubital vein in the amount of 80 to 90 mL and a flow rate of 4.0–5.0 mL/s (Iopromide/Ultravist® 370, Bayer HealthCare Pharmaceutical, Germany). A 50 mL bolus of normal saline was given after administering the contrast material. In a software, we used automatic peak enhancement detection in the descending aorta with the timing of the bolus using a threshold of +180 Hounsfield Units. Data acquisition was performed during a breath hold of approximately 8 to 10 seconds. Patients with heart rate > 70 bpm received 100 mg of metoprolol *per os* 1 h prior to examination.

Electrocardiography (ECG) was performed simultaneously with retrospective gating of the data, during the examination. Reconstruction was performed at 75% of the RR interval, with a slice thickness of 0.5 mm. The ECG was edited manually when the heart rate was irregular. Post-processing and evaluation were done on a workstation (Vitrea 1, Vital Images, USA), where all images were transferred.

All data were analyzed with post-processing tools such as multiplanar reconstructions (MPR), curved MPR (cMPR), maximum intensity projections (MIP) and volume rendering (VR) to three-dimensionally image of the complex anatomy of the coronary artery tree. Anomalies of origin and course, intrinsic coronary anomalies (myocardial bridging, aneurysms) and termination anomalies (fistulas) were checked.

Results

From January 2010 till December 2014 a total number of 1,562 patients were sent for evaluation of coronary arteries. Coronary anomalies were found in 45 (2.88%) patients. There were normal findings of the heart in 515 patients (32.97%). The other patients (64.15%) had coronary artery disease, stenting of coronary arteries or bypass operation (Table 1).

The most frequent coronary anomaly seen in our population group was absence of left main trunk with the separate origin of the left anterior descending artery (LAD) and left circumflex artery (LCx) originating from a left coronary sinus (LCS). This was found in 12 patients (an incidence of 0.77% or 26.7% of all coronary anomalies) (Figure 1).

In 10 (0.64%) patients intramural coronary artery (muscular bridge) was found, which most commonly involved proximal LAD (Figure 2).

Anomalous location of coronary ostium within the aortic root or near proper aortic sinus of Valsalva was found high in 7 (0.45%) patients and low in 3 (0.19%) patients (Figures 3 and 4).

Anomalous location of coronary ostium outside normal aortic sinuses in our study was present as right coronary

Table 1

Coronary artery anomalies detected in our series

Type of anomaly	Number of patients	Incidence (%)	Anomalies (%)
Absence of left main trunk – separate ostium for LAD and LCx	12	0.77	26.7
Bridging	10	0.64	22.22
High "take-off"	7	0.45	15.55
RCA from LCS	5	0.32	11.11
Low "take-off"	3	0.19	6.66
Aneurysms of coronary artery	2	0.13	4.44
LM from NCS	2	0.13	4.44
Coronary artery fistulae	2	0.13	4.44
Single coronary artery	2	0.13	4.44
Total	45	2.88	

LAD – left anterior descending; LCx – left circumflex; RCA – right coronary artery; LM – left main; LCS – left coronary sinus; NCS – non-coronary sinus.

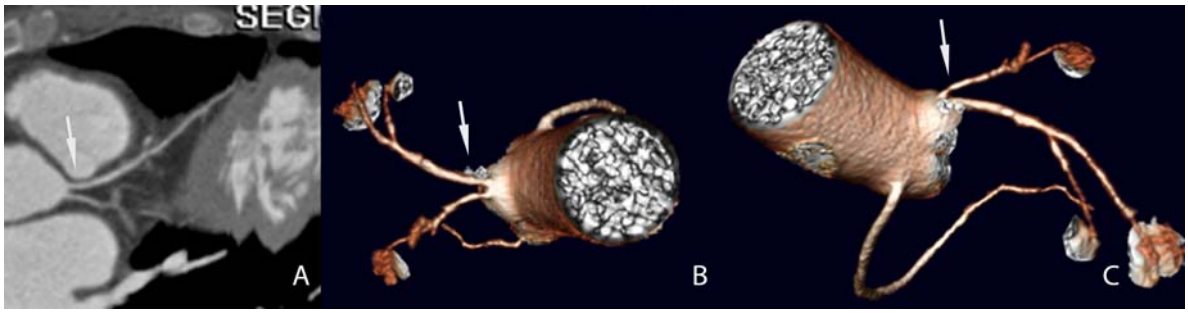

Fig. 1 – Absence of left main trunk with the separate origin of the left anterior descending artery (LAD) and left circumflex artery (LCx) originates from a left coronary sinus (LCS);
A) curved multiplanar reconstruction – MIP; B) and C) Volume rendering – VR.

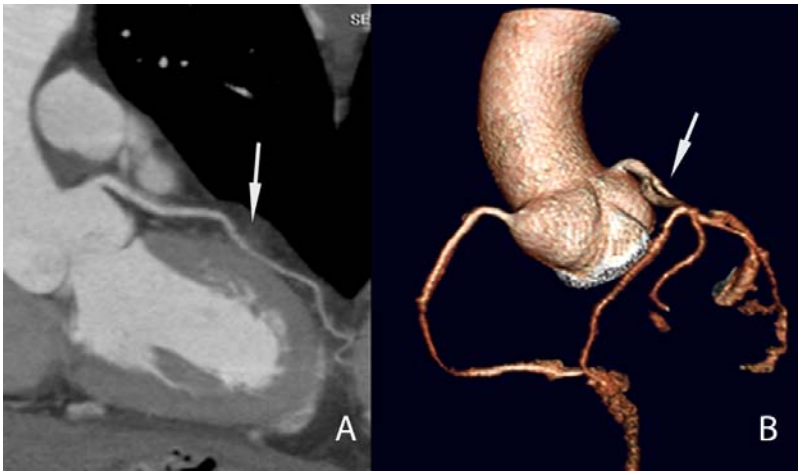

Fig. 2 – A) Maximum intensity projection (MIP), and B) 3D volume rendering show bridging of the left anterior descending.

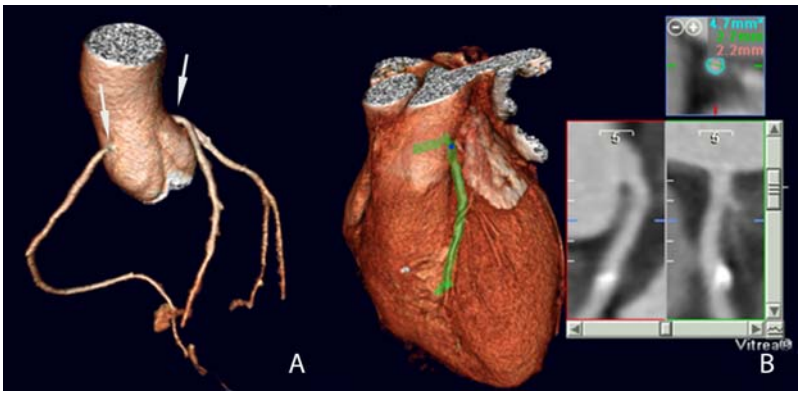

Fig. 3 – Volume-rendering images show high take-off of: A) the right, and B) left coronary arteries above the sino tubular junction.

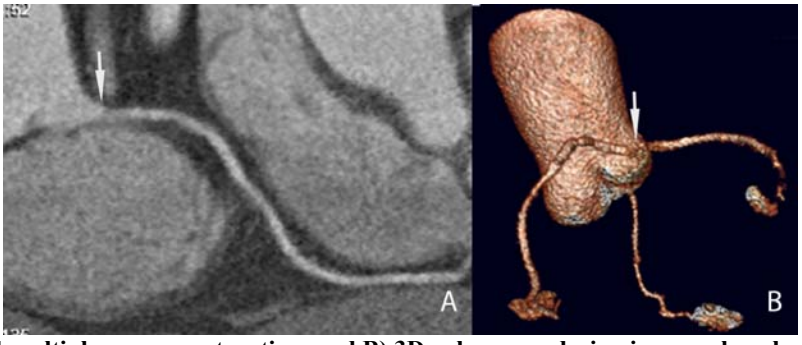

Fig. 4 – A) Curved multiplanar reconstruction, and B) 3D volume rendering images show low insertion of right coronary artery ostium.

artery (RCA) that arises from left anterior sinus in 5 (0.32%) patients and left coronary artery from non-coronary sinus in two (0.13%) patients (Figures 5 and 6).

The single coronary artery was found in two (0.13%) patients. One patient had a single coronary artery from the right coronary sinus and left coronary artery coming out of the RCA. The second one had single coronary artery from the left coronary sinus. A coronary artery fistula was found in two patients (0.13% or 4.44% of all coronary anomalies). The both of them had a fistula between the left anterior descending artery and the pulmonary artery (Figures 7–10).

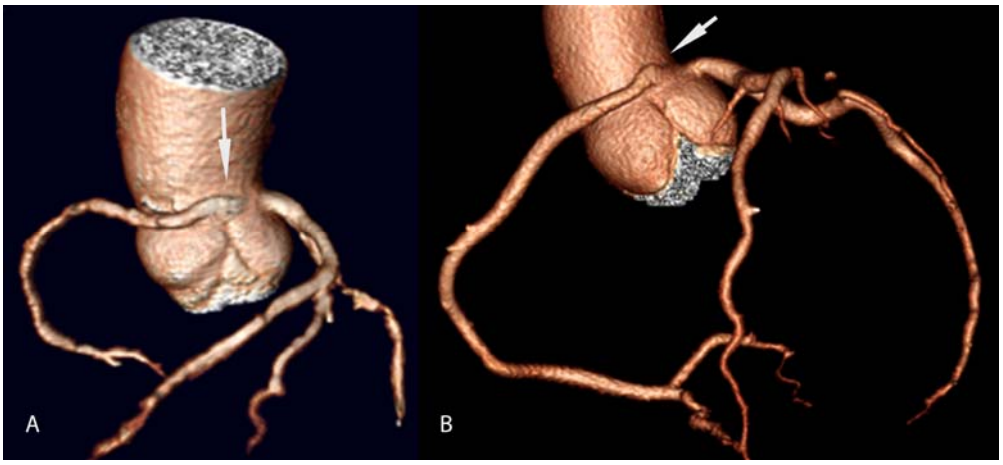

Fig. 5 – Volume-rendering images show two different patients with anomalous location of coronary ostium at improper sinus – right coronary artery arises from left coronary sinus.

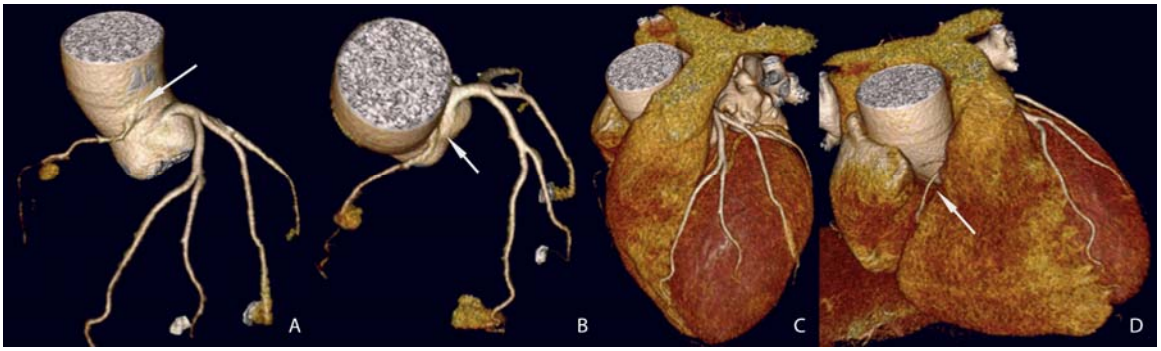

Fig. 6 – Volume rendering show right coronary artery arising from left anterior sinus, with anomalous course between aorta and pulmonary artery.

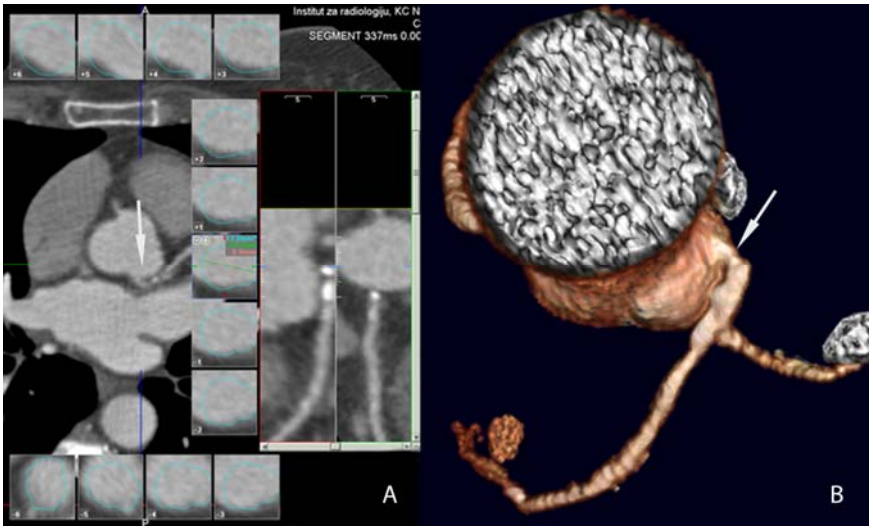

Fig. 7 – Left coronary artery arises from non-coronary sinus with angulation: A) curved multiplanar reconstruction; B) volume rendering images.

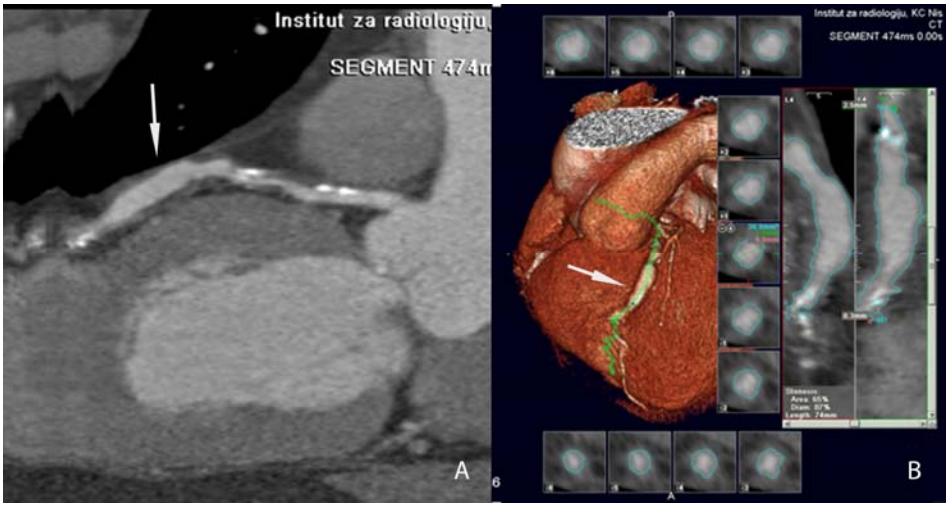

Fig. 8 – Aneurysm of left descending artery (LAD).

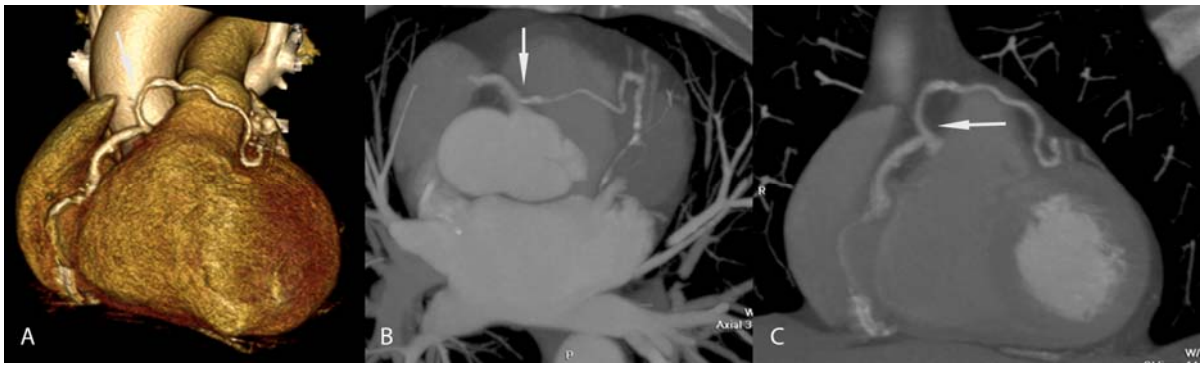

Fig. 9 – Single coronary artery arising from right coronary sinus – left descending artery (LAD) arises from the right coronary artery (RCA): A) volume rendering; B and C) multiplanar reconstruction.

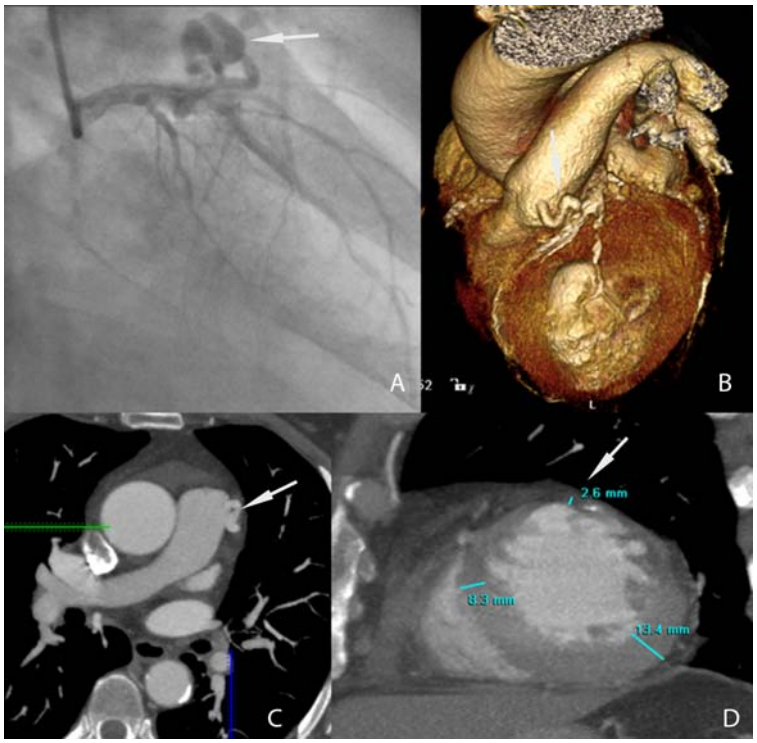

Fig. 10 – A) A fistula between the left anterior descending artery (LAD) and the pulmonary artery (PA); B) digital subtraction angiography (DSA) of LAD; C) Volume rendering show the fistula between PA and LAD; D) maximum intensity projection (MIP) axial view (short view of the heart with diameter reduction of myocard in the region of LAD vascularisation).

Discussion

Previously, congenital anomalies of the coronary arteries were distributed to the minor and major, depending on whether they may or may not cause relevant clinical consequences. However, as knowledge of the pathophysiology and clinical consequences of certain anomalies is often insufficient, this classification has been abandoned, and Angelini classification is increasingly being used, based on anatomical parameters (number, localization and size of the artery ostium, the angle at which they are separated, size of arteries, their flow in the proximal and medial segments, branching and termination) [1–3].

Dilemmas exist when it comes to the frequency of congenital anomalies of the coronary arteries. Earlier data from the literature indicates detection of these anomalies on autopsies and invasive coronary angiography. It has been found to occur less frequently than congenital heart disease, with an incidence about 1% of the population [4–9].

An invasive coronary angiography was traditionally used for the diagnosis of coronary anomalies [10, 11], until the appearance of the first publications in released statements in the multislice computed tomography.

On invasive angiography, it sometimes can be difficult to see a course of arteries, especially when it passes between the aorta and the pulmonary tree, thereby requiring specific projections and skillful interpretation. Three-dimensional reconstruction of coronary vessels on MSCT significantly facilitates observation of these anomalies.

Yamanaka and Hobbs [5] have published the largest invasive angiography series with 126,595 patients, and 1.3% incidence of the anomalous coronary arteries.

In our study, the incidence of coronary anomalies amounted 2.88% on MSCT angiography.

There is great diversity in the results of studies performed with multislice computed tomography. Graidis et al. [12] in their study analyzed 2,572 patients examined by MSCT and found 60 patients with coronary artery anomaly (incidence 2.33%).

In other study, anomalies were detected with the incidence of 2.5% in 44 patients out of the reviewed 1,758 [13].

Srinivasan et al. [14] assessed 1,495 patients using MSCT coronary angiography and found the prevalence to be 0.8 %.

Absence of left main trunk with the separate origin of LAD and LCx origin from a left coronary sinus in our study was found in 12 patients (an incidence of 0.77%). They are estimated to be seen in 0.5% to 8% of the population. This anomaly was the first most common anomaly in our study. Multiple arterial ostia [15] usually present no major clinical problems, but they may cause a problem during catheterization of the artery at invasive coronary angiography.

Anomalous location of coronary ostium outside normal aortic sinuses in our study was present like RCA that arises from left anterior sinus in 5 (0.32%) patients. The anomalies of the course of the coronary artery, especially when passing between the aorta and the pulmonary arteries, may be the cause of sudden cardiac death. Particularly people who are exposed to extreme physical exertion and athletes are at risk.

Coronary artery fistula is also within the anomalies that can cause serious complications in the heart or sudden cardiac death. Fistulas may occur as communication with the venous blood vessels or communication with coronary artery heart chambers. In our study, there was communication between the LAD and the pulmonary artery in two patients. One patient suffered a myocardial infarction and in the region of vascularization LAD and then the existence of fistula was discovered.

Myocardial bridging has a special place in the diagnostic examination of the coronary arteries on the MSCT. The possibility of assessing the condition of the coronary arteries in the myocardium as well as the length and depth of the segment is of particular importance. Having this information, further therapeutic treatment can be planned.

In our study muscular bridge was found in 0.64% of cases with most commonly involved LAD. There is some difference in the literature data between the prevalence of myocardial bridging at invasive angiography (0.5–2.5%) and prevalence at autopsy (15–85%) [16]. It can be explained by the fact that these patients often don't have any symptoms, so they do not undergo invasive angiography. In some patients myocardial bridging can cause angina pectoris, myocardial infarction, arrhythmias, or even death [17]. The main advantage of MSCT is the visualization of intramyocardial location of the coronary arterial segment. Relative limitation of this method is an image reconstruction using retrospective electrocardiography (ECG) gating in diastolic phase. If there is a suspicion for myocardial bridging, it is recommended to perform the ECG-gated reconstruction during the systolic phase as well as diastolic one.

Conclusion

Knowledge of the coronary artery anomalies and their recognition on the multislice computed tomography is of great importance for the further planning of a possible therapeutic treatment. Coronary anomalies that are treated as insignificant will require no further therapeutic treatment. But the detection of malignant coronary anomalies will certainly save many lives.

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The characteristics of family functioning with mentally ill children and adolescents

Karakteristike funkcionisanja porodica sa mentalno obolelom decom i adolescentima

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Abstract

Background/Aim. The family functioning and characteristics are the major risk factors in the genesis and persistence of mental disorders in children. The aim of this study was to evaluate the characteristics of functioning of family with mentally ill children and adolescents. **Methods**. This study explored 47 families with a child/adolescent suffering from mental disorders and 47 families of age matched healthy children/adolescents. The socio-demographic questionnaire, Social Adaptation Self-evaluation scale (SASS) and Family Adaptability and Cohesion Evaluation Scale (FACES III) (Olson, 1983) were completed by parents. **Results.** For all three FACES III dimensions multivariate analysis of variance (MANOVA) showed significant differences between groups (Wilks λ = .887; F = 3.839; df = 3; p = 0.012). Univariate analysis results showed significant differences for cohesiveness F = 6.99 p = 0.001 and adaptability F = 10.07 p = 0 .001. The analysis of the social adaption (SASS) assessment showed that the mean score for clinical vs. non-clinical group was 39.66 ± 6.82 vs. 38.06 ± 8.44 without significant difference between groups (p = 0.32). The families of mentally ill children showed frequently lower socioeconomic status and education level, higher number of children per family, and broken home. **Conclusion.** The results suggested that cohesiveness and adaptability were significantly more prominent among families with mentaly ill children, but adaptation was similar to families with healthy children. It would be useful to evaluate adaptability, cohesiveness and adaptation of primary families when planning prevention and rehabillitation of mentally ill children and adolescent.

Key words:
child; adolescent; mental disorders; parents; family.

Apstrakt

Uvod/Cilj. Prethodna istraživanja su ukazala na značaj osobina i funkcionisanja porodice kao važnih faktora rizika u nastanku i održavanju mentalnih poremećaja kod dece. Cilj ovog istraživanja je bila procena funkcionisanja porodica sa mentalno obolelom decom i adolescentima. **Metode**. Studijom je obuhvaćeno 47 porodica sa mentalno obolelim detetom/adolescentom koje su bile poređene sa 47 porodica sa zdravom decom/adolescentima. Roditelji su popunjavali Socio-demografski upitnik, Skalu socijalne adaptacije (*The Social Adaptation Self*-evaluation scale – SASS) i Skalu evaluacije porodične prilagodljivosti i kohezije (*Family Adaptability and Cohesion Evaluation Scale* – FACES III); Olson, 1983). **Rezultati**. Za sve tri dimenzije na FACES III skali multivarijantna analiza varijanse (MANOVA) je pokazala značajnu razliku između grupa (Wilks λ = .887; F = 3.839; df = 3; p = 0.012). Analiza varijanse (ANOVA) je pokazala da ta značajna razlika postoji za kohezivnost (F = 6.99 p = 0.001) i adaptibilnost (F = 10.07; p = 0.001. SASS analiza socijalne adaptacije je pokazala viši skor za kliničku vs. neklиničku grupu (39,66 ± 6,82 vs. 38,06 ± 8,44), bez značajne razlike između njih (p = 0.32). Porodice sa mentalno obolelim detetom/adolescentom češće imaju niži socioekonomski status, nezaposlenost roditelja, veći broj dece u porodici, i porodičnu separaciju (razvod). **Zaključak**. Rezultati su ukazali da su kohezivnost i adaptabilnost značajno uočljivi u porodicama sa mentalno obolelom decom, ali da je socijalna adaptacija slična porodicama sa zdravom decom. Bilo bi korisno da se pri planiranju prevencije i rehabilitacije dece /adolescentata sa mentalnim oboljenjem procene kohezivnost i adaptabilnost primarnih porodica.

Ključne reči:
deca; adolescenti; mentalni poremećaji; roditelji; porodica.

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Introduction

The functional family manages to adjust itself to changes and reorganizes while retaining its own identity and structure. The demand for changes within the family may come from the social surroundings (life events, sociocultural context) or from the family itself, that is, from the family members' needs for individual development in different stages of the family life cycle [1, 2]. Parental acceptance and rejection has a dramatic effect, especially when obseved by the individual, on children's personality and behavior as well as on the personality of an adult who considers himself to have been "a rejected child" [3]. Researches and clinical records support this supposition that rejection can interfere with a wide range of psychiatric illnesses and behavioral disorders including neurosis, schizophrenia, deliquency, psychophysical illnesses such as allergies, school problems, stammering and body dismorfic disorder [4]. A specific and particular form of parental care and communication with a child can be monitored over several generations. It happens that the abused children abuse their own children more often later in life [5]. There are also another factors that influence a child's behaviour such as: personal characteristics of parents, marital quarrels, and particular ways of upbringing [6]. These factors form the basis for a complex process of growing-up and they have an inevitable impact on child's behavior.

Researches conducted on families at the territory of Republic of Serbia indicate changes in family structure and functionality which are connected with broader social development and a process of transition. Apart from the changes in family patterns (single parent families emerging after divorce, casualties of war, and desire to be a sole parent), socioeconomical transition is also in connection with a reduced number of children per family, higher incidence of divorce, delay in getting married, but also the return of multi-generational family household [7–9]. According to the studies there has been a shift from a traditional family to an unbalanced family system and chaotic relations [10–12]. The importance of associations between characteristics of mentally ill children and their family was decsribed in many studies [13–15].

The aim of the present study was to evaluate the characteristics of functioning family with mentally ill children and adolescents.

Participants

The cross-sectional study was conducted in the Centre for Child and Adolescence Psychiatry at the Institute of Psychiatry in Clinical Centre of Vojvodina in Novi Sad and in health centers in Novi Sad and Bačka Palanka, from February 2014. to March 2015. In the clinical group the one parent from each family with a mentally ill child were consecutively recruited during regular control medical examinations of children. The 53 parents were enrolled, but 47 completed the study. The control group consisted of 47 parents of matched healthy children in health centers in Novi Sad and Bačka Palanka chosen during regular medical examinations.

The inclusion criteria were diagnosed mental illness of the children according to diagnostic criteria of the International Classification of Diseases, tenth revision (ICD-10) and parents age from 25 to 65 years. The criteria for exclusion of parents from the study were presence of neurological disorders; serious heart disease (fresh myocardial infarction), serious endocrine disorders, malignancies, substance abuse in previous 12 months and mental retardation.

The inclusion of parents was performed successively, according to inclusion and exclusion criteria, starting from the first day of testing onwards, up to the date when the total number of respondents was reached, in accordance with previous calculation of sample size.

Prior to entering the study, all participants signed informed consent and the survey was approved by the Ethics Committee of Clinical Center of Vojvodina.

Instruments

General questionnaire was designed for this study to collect the basic socio-demographic data obtained from the participants such as: gender, age, educational status, marital status, employment and material status of the family. Each questionnaire was completed by one parent.

Social Adaptation and Self Evaluation Scale (SASS) [16] consisting of 21 questions developed to detect the presumptive differences of social interactions, global social attitude, motivation and behavior. SASS focuses on a subject's self-perception and motivation focused on action rather than objective performance. It provides an understanding of an individual's level of satisfaction with his/her social situation. It evaluates the current situation, enjoying the activities of work, occupation and hobby, quality of leisure time, behavior in the family, the quality of family relationships, sociability, active social behavior, quality of relationships with people, evaluation of relations with the external environment, social attractiveness, social considerations, social embeddings, curiosity, intellectual preoccupations, difficulties in communication, a sense of rejection, vanity, the difficulty in managing income and environmental management. Twenty items are summarized for the total possible score of 60. In evaluation of overall results a higher score indicates better functioning [17].

Family Adaptation and Cohesion Scales (FACES III) is a questionnaire that assesses family adaptability and cohesion. [14] It investigates family dynamics and consists of 10 cohesion items and 10 adaptability items. The respondents indicate how frequently the described behavior occurred in his/her family on a Likert scale from 1 (almost never) to 5 (almost always). The total scores of adaptability and cohesion ranged from 10 points to 50 points, respectively.

Family cohesion assesses the degree of closeness or distance among family members on the basis of four stages: remote, separated, connected and networked. Adaptability was evaluated on four levels: family rigidity, structured, flexible and chaotic. The questionnaire consists of 20 questions in the form of a five-point scale; the sum of points on the uneven responses represent cohesiveness, and the sum of the even

numbers adaptability of the family. The Beavers system model, the importance of family competence, capability of the family (interaction units) to accomplish the tasks was set before. Competence is measured by Beavers interaction scale for competence (Beavers Interactional Competence Scale). Within circumscription model of marital and family systems there are three dimensions: family cohesion (emotional ties, internal boundaries, coalition, time, space, friends, decision making, interest and recreation), flexibility (leadership, control, discipline, arranging, styles, roles, relations, rules) and communication (listening skills, interview skills, inclusion, transparency, the ability to maintain the continuity of respect and regard for the caller) important to assess the functioning. Within Circumplex model, a high (chaotic) and very low (rigid) level of flexibility become problems for the individual and the relationship, if long lasting. Relations with the average score (structured and flexible) achieve stability and the possibility of applying a functional way [3].

Statistics

The descriptive statistics was applied with the absolute and relative numbers; measures of central tendency (mean, median) and measures of dispersion (standard deviation, variation interval) From parametric and nonparametric tests analysis of variance of repeated measurements, the Friedman's test and the Wilcoxon's test were used, respectively. The Pearson's and Spearman's tests were used for testing correlations, while in certain situations for testing connectivity dynamics of the two parameters linear mix model was applied. All data were processed in SPSS 20.0 software package. The differences with $p < 0.05$ were considered significant.

Results

Sociodemografic characteristics

Among parents, more mothers (73.4%), and more children and adolescents of male gender were registrated (58.5% vs. 41.5%). Age of parents ranged from 26–57 years, and children from 4 to 17 years. There were no statistically significant differences between the clinical and the control group in the level of parental education. In the clinical group significantly more parents were unemployed.

Regarding marital status in the control group significantly greater number of married subjects was observed ($p \leq 0.05$), while the percentage of those in common-law marriage and divorced ones was significantly lower.

Statistically significant difference between the clinical and non-clinical (control) group was observed regarding their financial status ($p = 0.05$) in terms of higher prevalence of families with below-average financial status in the clinical group.

There is a statistically significant difference ($p \leq 0.05$) between the clinical and the non-clinical group regarding the number of children in a family, with a greater number of children in clinical families than in non-clinical ones.

The most frequent mental disorders among children were behavior disorders (28%), followed by emotional disorders (approximately 19%) and psychotic disorders (15%) in the clinical group.

The mean scores for the clinical vs. non-clinical group were assessed regarding cohesiveness (42.02 vs. 35.17) and adaptability (38.79 vs. 31.23). Univariate analysis results showed significant differences for cohesiveness $F = 6.99$ $p = 0.001$ and adaptability $F = 10.07$ $p = 0$ .001.

For all three FACES III dimensions MANOVA was performed and significant differences between groups were registered: Wilks $\lambda = 0.887$; $F = 3.839$; $df = 3$; $p = 0.012$ (Figure 1).

The analysis of the social adaption assessment (SASS) showed that the mean score and standard deviation (SD) for clinical vs. non-clinical group was $39{,}66 \pm 6{,}828$ vs. $38{,}06 \pm 8{,}445$, respectively, and there was no statistically significant difference between groups ($t = 1{,}007$ $df = 92$; $p = 0.32$) (Figure 2).

Discussion

In this study, the sociodemografic characteristics and influence of children's mental disorders on family functioning, primarily on adaptability and cohesion were investigated.

The results showed that there were significantly less frequent two-parents families and a common-law marriage and separated couples were more frequent in the families with mentaly ill children compared to families with haelthy children. During last decades the proportion of children in two-parent families decreased and high divorce rate in population in general was recorded, so overall trends suggest that more than one quarter of all children live with a single parent, usually with their mother [18]. In our study there were more mothers than fathers (three quarter vs. one quarter of participants) and male children were more frequent. It is in concordance with earlier reports in which a comparable impact of mother-child attachments has been shown [19].

There were no significant differences between the clinical and the control group in the parents' education level. However, in the clinical group below average financial level was more prevalent and more parents were unemployed. It was in concordance with previous research in which family risk factors for children psychopathology included low socioeconomic status, large family size and divorce [20]. Also, the greater number of children (three or more) per family was more frequent in the clinical group. The changes in family structure and children's health are strongly related to family income and the financial resources, but parenting may moderate risk effects [18]. In families with more children, parents are more burdened, which may be a risk factor for development of a child's behavior disorder and a large number of children is more frequently connected with lower socio-economic status. A low income could have a negative effect on parental skills as well as on a child, which creates the potential for family violence, neglect and abuse [21]. A key component of the experience of early childhood poverty may be of a high level of cumulative risk exposure, especially consequential for children's psychological well-being [22].

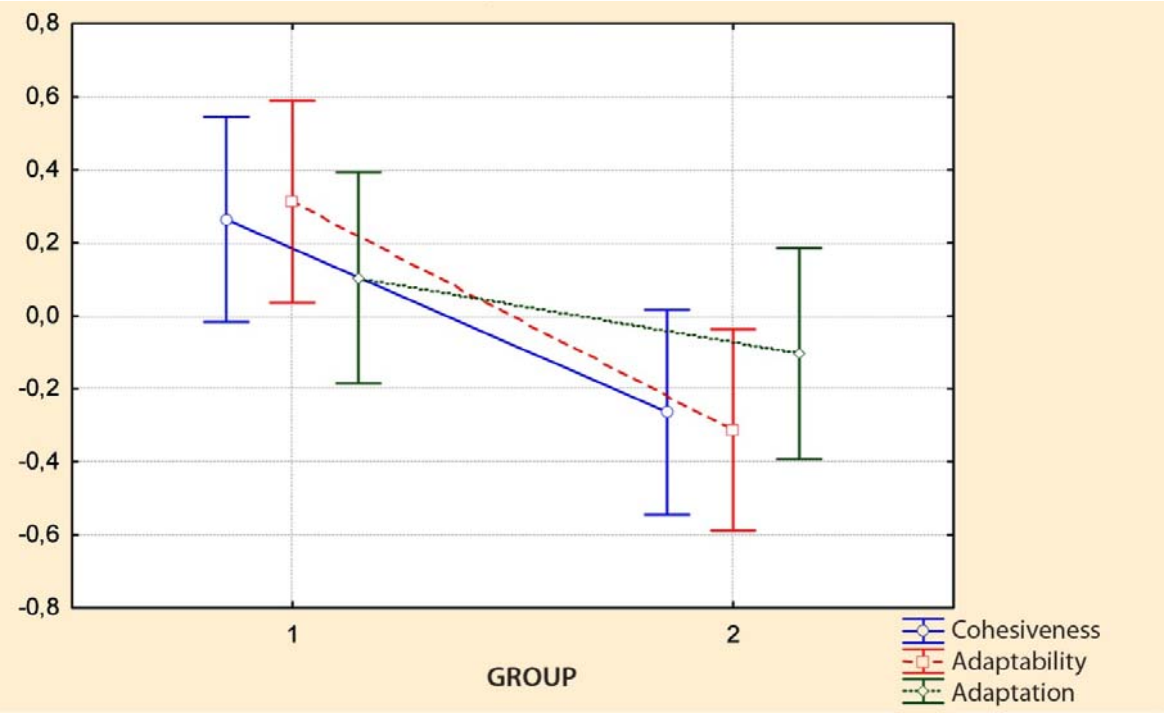

Fig. 1 – The differences between groups regarding dimensions on the Family Adaptation and Cohesion Scale (FACES III)
Group 1 – clinical group (families with mentally ill children)
Group 2 – non-clinical group (families with healthy children)

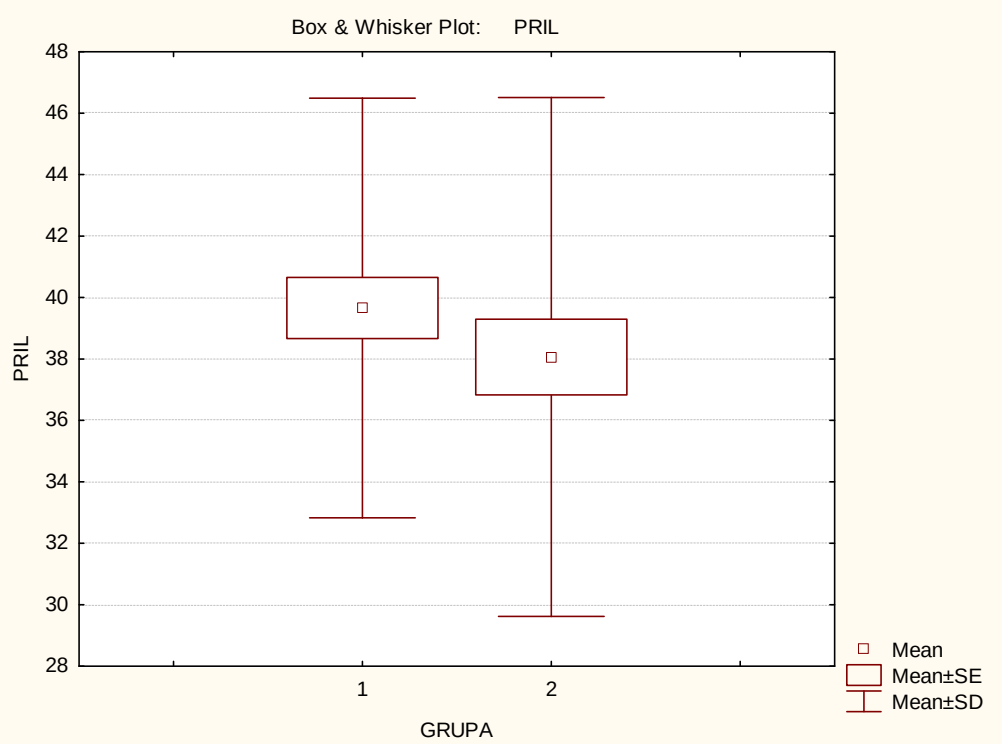

Fig. 2 – The differences between groups regarding Social Adaptation and Self Evaluation Scale (SASS)
Group 1 – clinical group (families with mentally ill children)
Group 2 – non-clinical group (families with healthy children)
SE – standard error; SD – standard deviation.

In this paper the most frequent mental disorders among children in the clinical group were behavior disorders (28%), followed by emotional disorders (approximately 19%) and psychotic disorders (15%). However, the results from prior studies showed similar characteristics of the family over a broad range of child psychopathologies. So, conduct problems, aggression symptoms and depression were uniquely associated with specific family environments marked by less cohesiveness, greater conflict and intellectual/cultural pursuits [23].

An unfavorable family atmosphere, unstable family relationships and parental vulnerability to stress are recognised as risk factors for development of mental disorders in children [15]. Studies in this area indicate that beyond type of disability, child's self-regulatory processes and family climate, especially mother-child interaction were key predictors of change in both parent well-being and child development [24]. There are suggestions that for an individual personality development parental influence is crucial and a good parent–child relationship may promote children's behavioural and emotional resilience to multiple environmental risk exposure [25].

There was no difference between the clinical and the non-clinical group regarding parental social adaption. This was unexpected if we take into account that there was a high incidence of unemployment, divorce, lower socioeconomic status as well as that these parents take care of a child with a mental disorder. This partly could explained by parents' compensatory mechanisms and skillfulness in struggling with a child's mental disorder and partly by the symbiotic relationship of parents (especially mothers) with ill child, which comes as a consequence [21].

The measures of family functioning showed difference between the clinical and the non-clinical group. The cohesiveness and adaptability significantly were higher in the clinical group. compared with the non-clinical group. It might be unexpected, because, according to the Beavers systems model, the families where a certain mental illness emerge, are less adaptable, more rigid, while this survey showed the opposite situation [26]. In many previous studies, it was reported that adolescent problem behaviors are related to family functioning [27]. The family cohesion has significant impact on psychiatric symptoms, but the stronger associations for adolescent ratings than parental ratings exist [28].

Education level of parents, socioeconomic status, number of siblings, residential area, and other factors can influence family adaptibility [29].

According to the results there was a greater degree of interaction in families with children and adolescents who suffer from mental disorder than in families from the control group. These unexpected results might be the consequence of a tendency of parents to present themselves in a socially desirable context [3, 20]. Presumably, as a reaction to a child's illness, family uses higher cohesion, more care, mutual support and interaction as a strategy [16]. Cohesiveness and adaptability which are optimally develop can preserve the family structure with successfully overcoming expected and accidental life events [24]. The lack of cohesiveness and conflicts in the family may predict unfavourable development of children [30]. The factors which influence mental health can be divided into high-risk factors and protection factors and could be targets for intervention.

Various aspects of family relations and the atmosphere in the family may be predictive for social skills achivement of a child [26]. Interventions should be sensitive to the stages of child and adolescents development and should promote family communication taking into consideration social and cultural differences [31]. Multidisciplinary approach is necessary for realization of the objectives and their effect.

The present results were consistent with previous research which showed that family functions and adolescent problem behaviors do not have a curvilineaer relationship [28]. It was reported from previous study that family adaptability can be affected by socioeconomic education level of parents, number of siblings, financial status, residential areas and some other factors [27, 29]. Thus, the higher mean score for family adaptability in our study might be explained by high education level of parents.

Social adaptation and attachment are partly overlapping due to their shared social nature. Attachment style develops by early parent-child interactions and demonstrates relative stability [32]. It is very important to ensure an early relationship between a parent and a child in order to establish optimal functioning in various life segments. High-risk and protection factors can be targets for family-focused intervention with consideration of a culturally specific factors that may promote family communication about mental health [31]. There are several limitations to the present study: it is cross- sectional in nature with a relatively small number of respondents from a narrow territory and only one parent completed the questionnaires. It would be interesting to compare evaluations of family functioning carried out by children suffering from a mental disorder as well as both their parents. Despite these limitations, the results of this study might provide useful information related to functioning of families with mentally ill children and may serve in counseling and treating children in clinical settings.

Conclusion

The present study found that families with mentally ill children had more frequent parental separation, more children per family, higher rate of unemployment of parents and lower socioeconomic status. There were more prominent cohesiveness and adaptability among families with mentaly ill children, but adaptation was similar to families with healthy children.

This findings suggest that it would be useful to evaluate adaptibility, cohesiveness and adaptation of primary families when planning prevention and rehabilitation of mentally ill children and adolescents.

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The effects of different caloric restriction diets on anthropometric and cardiometabolic risk factors in overweight and obese females

Uticaj različitih kalorijskih ograničenja u ishrani na antropometrijske i kardiometaboličke faktore rizika kod predgojaznih i gojaznih žena

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Abstract

Background/Aim. Obesity is an established risk factor for numerous chronic diseases. The aim of this study was to investigate the effect of well-balanced different caloric restriction (CR) diets on anthropometric parameters and standard biochemical cardiovascular risk markers [lipid profile, glucose homeostasis and high sensitivity C-reactive protein (hs-CRP)] in overweight/obese females. **Methods.** Participants (age 20–40 years) were randomized into 3 different CR diet groups: the group I – restriction of 20% calories from baseline energy requirements, the group II – restriction of 50% calories from baseline energy requirements and the group III – alternating daily diets with 70%/30% restriction. The study lasted 42 weeks. Anthropometric parameters were measured at the start and after 4, 8, 20 and 42 weeks after dietary intervention beginning. Biochemical markers were determined at baseline and after 20 and 42 weeks from dietary restriction start. **Results.** Body weight, body mass index (BMI), waist circumference (WC) and body fat (in %), in the different CR diet groups significantly decreased after 42 weeks. Body weight was less 11 kg in the group I and 12 kg in the groups II and III. WC was reduced by 11 cm in the groups I and III and by 10 cm in the group II. Different CR diets had the same effects on body fat (a reduction of 15% of body fat). Total cholesterol decreased by 7% in the group I and by 8% in the group III. Low density lipoprotein (LDL) cholesterol decreased by 14% in the group I and by 13% in group III. There were no significant changes in total and LDL-cholesterol levels in the group II. The atherogenic index presented as trigliceride/high density lipoprotein (TG/HDL) ratio decreased by 0.22 in the group I, by 0.25 in the group II and by 0.32 in the group III. Various CR diets had the same effects on reducing the hs-CRP levels. **Conclusion.** Different CR diets with the same macronutrient content are equally effective in reducing body weight, WC and body fat, improve cardiometabolic risk factors and decrease level of pro-inflammatory hs-CRP in overweight/obese females.

Key words:
diet, reducing; anthropometry; obesity; women; lipoproteins; glucose; c-reactive protein; body mass index.

Apstrakt

Uvod/Cilj. Gojaznost je faktor rizika od nastanka mnogih hroničnih bolesti. Cilj istraživanja bio je da se ispita efekat dobro izbalansirane ishrane različitih kalorijskih ograničenja na antropometrijske parametare i standardne biohemijske kardiovaskularne markere rizika [(lipidni profil, homeostazu glukoze, visoko senzitivni C-reaktivni protein – high-sensitivity C-reactive protein (hs-CRP)] kod predgojaznih/gojaznih žena. **Metode.** Ukupno 97 žena, između 20 i 40 godina starosti, konzumirale su uravno-težene, kalorijski različite-restriktivne dijete, na sledeći način: I grupa (n = 37) – ograničenje 20% kalorija od osnovnih energetskih potreba; II grupa (n = 30) – ograničenje od 50% kalorija od osnovnih energetskih potreba i III grupa (n = 30) – naizmenična dnevna restrikcija od 70% i 30% kalorija od osnovnih energetskih potreba. Stepen uhranjenosti je određivan antropometrijskim merenjima na početku i nakon 4, 8, 20 i 42 nedelje od uvođenja dijeta. Biohemijski markeri analizirani su na početku i nakon 4, 8, 20 i 42 nedelje. **Rezultati.** Kalorijski različite restriktivne dijete dovele su do značajnog smanjena telesne mase, indeksa telesne mase (*body mass index* – BMI), obima stuka (OS) i % telesne masti nakon tretmana

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od 42 nedelje. Telesna masa snižena je za 11 kg u grupi I, a za 12 kg u grupama II i III. Za 11 cm je smanjen OS u grupama I i III i za 10 cm u grupi II. Gubitak 15% ukupne telesne masti ostvaren je za sve vrste dijeta nezavisno od različitog kalorijskog unosa. Koncentracija ukupnog i (*low density lipoprotein*) holesterola (LDL-holesterola) snižena je za 7% i 14% u grupi I i za 8% i 13% u grupi III. Dijeta sa redukcijom kalorijskog unosa od 50% nije imala efekta na nivo ukupnog i lipoprotein niske gustine LDL-holesterola. Aterogeni indeks predstavljen kao odnos trigliceridi/lipoprotein visoke gustine (*high density lipoprotein*) – TG/HDL bio je manji za 0,22 u grupi I, za 0,25 u grupi II i za 0,32 u grupi III. Kalorijski različite restriktivne dijete dovele su do istog sniženja nivoa hs-CRP. **Zaključak.** Restriktivne vrste dijeta sa različitim kalorijskim unosom i sa istim procentom zastupljenosti makronutrijenata jednako su efikasne u smanjenju telesne mase, OS i % telesne masti, dovode do poboljšanja faktora kardiometaboličkog rizika i smanjenja proinflamatornog hs-CRP kod predgojaznih/gojaznih žena.

Ključne reči:
dijeta, redukciona; antropometrija; gojaznost; žene; lipoproteini; glukoza; c-reaktivni protein; telesna masa, indeks.

Introduction

Increasing obesity is declared as a huge public health problem in the world. Some of chronic diseases are closely related to the existing obesity. These are atherosclerosis, hypertension, hyperlipidemia, insulin resistance syndrome [1, 2], musculoskeletal diseases, polycystic ovary syndrome, some types of cancer and lack of a sense of psychological well-being. Serbia has a growing population of overweight people in the last ten years, too [3]. In fact, about 35% of adult population are overweight and 21% considered obese [4]. Metabolic risk factors are closely related to nutrition status and body composition. Inflammation and cardiovascular diseases are result of obesity [1, 5].

Waist circumference (WC) is most commonly used as an indicator of abdominal adiposity [6]. Obesity with a combination of abdominal obesity is related to metabolic disorders such as dyslipidemia and hyperinsulinemia, disrupted homeostasis and inflammation [2, 5]. Nutritional therapy is a sovereign method in achieving weight loss. [7] Calorie restrictive (CR) diets with energy limitation and balanced macronutrient composition are most often applied [8, 9]. The success in achieving weight loss is determined with degree of calorie restriction.

Calorie restrictive diets consist of reducing energy intake by 15–60% of common daily energy requirements. Obesity treatment guidelines issued by the National Institute of Health considered a good reduction in caloric intake by 500 kcal/day in the treatment of overweight and the class I obesity. This level of CR diet is recommended to people with diagnosed more than one risk factor, too [10].

Low-calorie diets (LCDs) are high in carbohydrate (55–60%) and low in fat (less than 30% of caloric intake). Also, LCDs have a low glycemic index, but a high fiber content. There is a solid evidence that indicates that LCDs during a period of 3–12 months, can lower total body weight by an average of 8% [10, 11]. Thus, it is implicated that CR may be critical factor in determining the results of intervention.

Different dietary interventions with weight loss, decrease of abdominal obesity, an achievement of desirable values of serum cholesterol, triglycerides and fasting insulin concentrations glucose tolerance and blood pressure as a goal have been set in the last decades [12–14].

To our knowledge, the number of studies that have examined the influence of different dietary intervention on enhancing weight loss and reducing cardiometabolic risk factors including blood lipids profile, glucose homeostasis and inflammation are limited. Therefore, the aim of this study was to investigate the long-term effects of well-balanced diet with different CR on anthropometric parameters, body fat mass and standard biochemical cardiovascular risk markers, e.g., lipid profile, parameters of glycoregulation and high-sensitive C-reactive protein (hs-CRP) in overweight and obese females.

Methods

Participants

We designed a follow-up, prospective, intervention study in the routine management of 240 overweight or obese females that attended the Department of Nutrition, Institute of Hygiene, Military Medical Academy (MMA) and were initially enrolled from January 2014 to May 2015. Due to the high drop-off rate, 97 females with baseline body mass index (BMI) $\geq$ 25 kg/m^2, completed the study.

Key inclusion criteria were: female gender**,** age 20–40 years, BMI between 25 and 44.9 kg/m^2, stable weight (± 2 kg) and sedentary or lightly active status for 3 months before beginning of the intervention, with normal fasting glucose levels. Subjects that had history of cardiovascular disease, liver or kidney dysfunction or cancer, taking weight loss, lipid-lowering, or glucose-lowering medications were excluded. The study was approved by the Human Research Ethics Committee of the MMA. Written consent was signed by all study participants at the start of the study.

Study design

Participants were matched for age and BMI, and then were randomly selected using computer-generated random number allocation for consumption of different well-balanced caloric restriction diets during 42 weeks.

Participants were selected into one of three groups: the group I (n = 37) – restriction of 20% calories from baseline energy requirements; the group II (n = 30) – 50% CR from baseline energy requirements and the group III (n = 30) – 70%/30% CR alternating daily diets (one day 70%

and the following day 30% caloric restriction, respectively from baseline energy requirements). Participants in the CR groups were educated by our medical dietitian on principles to reduce their daily energy intake in order to achieve weight reduction.

After careful consideration of global nutrition, a personalized CR diet (700–1700 kcal/day) was prescribed, aiming at the achievement of a moderate weight loss. These low and moderate calories diets are based on conventional-type of intervention. In general, diets are low in energy and fat caloric intake with high carbohydrate and fibre content, based on an increased intake of whole grain cereals, vegetables and fruit [10]. Proposed food plan included carbohydrates (55–60% of total calories with 14 g dietary fibre/1,000 kcal), proteins (15–20% of total calories with 1.0 g/kg body weight/day), dietary cholesterol lower than 300 mg/day and limit of fat intake to < 30 % of total calories (with limit of saturated fatty acid to < 10 % of total calories intake) mostly mono and polyunsaturated fats. [10, 11] The CR diets were also balanced in terms of micronutrients intake (vitamins and minerals in the recommended diet must always be checked). On a very LCDs, weight losses for women have been reported to be 1.5–2 kg/week with the average weight loss of 20 kg during 12 weeks. Low-energy diet for 20–24 weeks, lead to reduce of 0.4–0.5 kg/week with the average weight loss of 8.5 kg. [15] There was no special physical activity prescribed during the study. Individuals met with our medical dietician at baseline, and after 4 and 8 weeks and later were followed monthly. The initial weight-loss goal was expected to be 10% over during 10-month dietary treatment period.

Anthropometric parameters

Anthropometric parameters were measurement at baseline and after 4, 8, 20 and 42 weeks in three different CR groups. A qualified nutritionist conducted anthropometric measurements. Body weight and height were measured and BMI was calculated. The height and weight of the participants dressed in light clothing without shoes were determined. Standard measurements of body height were performed. Obesity was classified, according to the World Health Organization (WHO) guidelines. [5, 10] Percentage of body fat was calculated from thickness of skinfolds on 4 measured points [16]. After measurements of WC subjects were classified into 3 groups according to health risk [17]. Anthropometric measurements were taken by one person.

Biochemical analyses

At baseline, after 20 weeks and at the end of dietary treatment (week 42), fasting blood samples were collected from all participants. The serum concentration of glucose, hemoglobin glucosylated test (HbA1C), triacylglycerols (TG), total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol and hs-CRP were measured using enzymatic kits (*Roche Diagnostics, Basel, Switzerland*) on a Siemens autoanalyser (*Dimension®, RxL Max, Siemens Dade Behring*). Calculations mde were: low-density lipoprotein (LDL) cholesterol via the Friedewald formula, [18] and atherogenic index as TG/HDL cholesterol ratio. We established a cut-off value of 5% at 6 months to indicate the efficacy of treatment.

Statistical analysis

Anthropometric parameters and concentrations were presented as mean values ± standard deviation (SD) and statistically analyzed by SPSS 20 software (IBM, Armonk, NY, USA). Since all variables showed normal distribution, checked by Shapiro Wilk test, univariate analysis of variance (ANOVA) was performed to assess differences in baseline characteristics and dietary data among CR groups. The effect of a diet duration (time 0–42 weeks) and the diet itself (different CR treatments) on continuous variables was determined using repeated ANOVA (taking time as within-subject factor and a diet as between-subject factor) to assess differences among different CR diet groups during the study (effect: time x CR group). *Post hoc* analyses were applied with Bonferroni adjustments for multiple parameters comparisons. Differences among groups were assessed by repeated ANOVA, followed by Tukey *post hoc* test. P values < 0.05 indicated statistical significance.

Results

Two hundred and forty subject were assessed at the begining of the study (week 0) and 97 subject (the group I – 20% CR, n = 37, the group II – 50% CR, n = 30, the group III – 70/30% CR, n = 30), who represent approximately 40% of the participants from the beginning of the study, completed dietary interventions after 42 weeks. At the beginning , various CR diet groups were similar in most parameters that were monitored, except WC, serum fasting glucose and hs-CRP levels, which were significantly lower in the group III with 70%/30% alternating daily CR compared to baseline levels in the group I – 20% CR and the group II – 50% CR (Table 1).

During the study period of 42 weeks, different CR groups had similar reductions in body weight, percentage of body fat and WC (time 0–42 weeks: $p < 0.001$; effect: time x CR diet group; $p > 0.05$) (Table 1). All CR-diet groups achieved a weight loss of 5% of their initial body weight after 4 weeks of the treatment. Waist circumference decreased ($p < 0.001$) by 11 cm in the group I (20% CR) and by 12 cm in the group II (50% CR) and the group III (70%/30%) from initial values during weight loss period, despite that WC was low at baseline in the group III (70%/30%) compared to other two CR groups. Different caloric restriction diets had the same reduction of 15% of total body fat compared to baseline (different treatments $p = 0.432$, time 0–42 weeks: $p < 0.001$). Table 1 shows that during treatment period, BMI in different CR diet groups

Table 1

Antropometric parameters and body fat before and after 4, 8, 20 and 42 weeks consummation of different calorie restrictive (CR) diets

Parameters	Group I – 20% CR (n = 37)	Group II – 50% CR (n = 30)	Group III – 70%/30% CR (n = 30)	Different treatment [c] (probability)	Time interval 0–42 weaks [d] (probability)	Effect Time x CR group [e] (probability)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$			
Age (years)	31 ± 8	32 ± 8	32 ± 7			
Height (cm)	167 ± 6	166 ± 6	168 ± 5			
Weight (kg) by week						
0	84.70 ± 13.52	85.62 ± 11.87	82.73 ± 10.99			
4	80.49 ± 13.38	81.38 ± 11.89	78.41 ± 10.85			
8	78.30 ± 12.93	78.59 ± 11.39	75.77 ± 10.41	$p = 0.047$ [c]	$p < 0.001$ [d]	$p = 0.002$ [e]
20	75.50 ± 12.90	75.35 ± 11.07	73.10 ± 10.25			
42	73.67 ±12.09	73.29 ± 10.98	70.84 ± 10.18			
Weight changes (kg) by week intervals						
0–4	-4.21 ± 2.61	-4.23 ± 1.19	-4.32 ± 1.83			
0–8	-6.70 ± 3.08	-7.23 ± 2.31	-6.96 ± 2.38			
0–20	-9.20 ± 7.91	-10.26 ± 5.60	-9.63 ± 3.78			
0–42	-11.03 ± 3.64	-11.93 ± 5.55	-11.89 ± 4.06			
BMI (kg/m^2) by week						
0	30.19 ± 3.93	30.93 ± 4.39	29.29 ± 3.79			
4	28.67 ± 3.92	29.39 ± 4.37	27.76 ± 3.74			
8	27.88 ± 3.79	28.38 ± 4.19	26.83 ± 3.60	$p = 0.257$ [c]	$p < 0.001$ [d]	$p = 0.473$ [e]
20	26.44 ± 4.08	27.23 ± 3.14	25.88 ± 3.56			
42	26.24 ± 3.60	26.49 ± 4.14	25.05 ± 3.50			
BMI changes (kg/m^2) by week intervals						
0–4	-1.52 ± 0.91	-1.54 ± 0.46	-1.54 ± 0.63			
0–8	-2.31 ± 1.05	-2.55 ± 0.79	-2.46 ± 0.83			
0–20	-3.75 ± 2.89	-3.60 ± 1.99	-3.41 ± 1.34			
0–42	-3.95 ± 1.23	-4.44 ± 1.96	-4.74 ± 1.61			
WC (cm) by week						
0	96.82 ± 10.46**	95.88 ± 13.29*	91.03 ± 10.70			
4	92.04 ± 11.293	93.63 ± 12.18	86.92 ±10.24			
8	92.01 ± 10.52	91.12 ± 11.81	84.80 ± 9.97	$p = 0.046$ [c]	$p < 0.001$ [d]	$p = 0.637$ [e]
20	88.55 ± 10.36	87.87 ± 10.95	81.85 ± 9.04			
42	85.78 ± 16.94	85.51 ± 10.80	80.30 ± 9.34			
WC changes (cm) by week intervals						
0–4	-4.78 ± 2.86	-2.25 ± 2.86[b]	-4.11 ± 3.02			
0–8	-4.81 ± 4.67	-4.75± 6.73	-6.23 ± 3.68			
0–20	-8.27 ± 4.62	-8.01 ± 6.84	-9.18 ± 5.18			
0–42	-11.04 ± 6.52	-10.37 ± 8.65	-10.73 ± 5.63			
Body fat (%) by week						
0	41.46 ± 4.72	41.33 ± 5.55	39.72 ± 4.34			
4	40.09 ± 4.46	39.88 ± 5.23	38.51 ± 4.65			
8	38.36 ± 4.04	37.88 ± 4.44	36.99 ± 4.66	$p = 0.423$ [c]	$p < 0.001$ [d]	$p = 0.432$ [e]
20	36.98 ± 4.34	36.26 ± 5.00	34.89 ± 4.93			
42	35.43 ± 5.08	34.76 ± 4.59	33.68 ± 5.17			
Body fat changes (%) by week intervals						
0–4	-1.37 ±1.25	-1.45 ± 1.93	-1.21 ± 2.18			
0–8	-3.20 ± 5.62	-3.45 ± 3.20	-2.73 ± 2.50			
0–20	-4.48 ± 2.55	-5.07 ± 3.44	-4.83 ± 2.70			
0–42	-6.03 ± 3.76	-6.57 ± 4.20	-6.04 ± 4.07			

Abbreviations: * $p < 0.05$ ** $p < 0.01$ [significantly different to the group III (30%/70%) CR] (univariate ANOVA); [a] $p < 0.05$ [b] $p < 0.01$ [Comparison of baseline characteristics at week 0 among groups (one-way ANOVA)]. Comparison of different CR treatments (repeated ANOVA) [d] Comparison of changes over time among groups from week 0, to 4, 8, 20 and 42 (repeated ANOVA)
[e] Comparison of treatment effect among groups for the changes from week 0, to 4, 8, 20 and 42 (repeated ANOVA).
BMI – body mass index; WC – waist circumference.

decreased significantly by 3.95 kg/m^2 in the group I (20% CR), by 4.44 kg/m^2 in the group II (50% CR), and by 4.79 kg/m^2 in the group III (70%/30% CR). After 42 weeks, total cholesterol decreased by 7% (change -0.35 mmol/L) in the group I (20% CR) and by 8% (change – 0.43 mmol/L) in the group III (70%/30% CR) compared to baseline. LDL-cholesterol decreased by 14% (change -0.47 mmol/L) in the group I (20% CR) and by 13% (change -0.42 mmol/L) in the group III (70%/30% CR) compared to the baseline levels (Table 2). There was no significant changes in the total and LDL-cholesterol serum levels during the study period in the group II (50% CR). However, after 42 weeks the serum HDL-cholesterol level decreased significantly ($p < 0.049$) only in the group I (20% CR) while the 70%/30% CR diet had no effects on serum HDL-cholesterol levels in our study participants. Atherogenic index presented as TG/HDL ratio was significantly decreased by 0.22 ± 0.23 in the group I (20% CR), by 0.22 ± 0.29 in the group II (50% CR), and by 0.32 ± 0.28 in the group III (70%/30% CR).

Table 2 shows that after 42 weeks the level of the serum HDL-cholesterol increased significantly by 0.07 mmol/L, serum triglycerides levels decreased by 0.25 mmol/L, total and LDL-cholesterol decreased by 0.35 mmol/L and 0.47 mmol/L respectively, plasma levels of fasting glucose decreased by 0.12 mmol/L, concentration of $HbA1_C$ decreased by 0.25% and hs-CRP levels decreased by 2.22 mg/L in the moderate caloric restriction diet group (the group I). Repeated ANOVA showed significant differences in the serum total and LDL-cholesterol levels from baseline to the end of the study when compared treatment effect among CR groups (effect: time x CR group: $p < 0.025$ and $p < 0.024$, respectively). The diet with 20% energy deficit had no significant effect on total and LDL-cholesterol levels in the serum but only in this diet group fasting glucose levels decreased significantly (different treatment: $p < 0.012$). Also, this diet group had the greatest reduction in $HbA1_C$ by 0.45% considering the baseline levels, compared to other two CR groups according to the treatment duration (time 0–42 weeks: $p < 0.001$). The highest decrease in triglycerides (0.38 mmol/L) and smaller reduction in levels of fasting glucose (0.09 mmol/L) was in the diet group III with 70%/30% alternating daily CR. HDL-cholesterol concentration in this diet group, firstly decreased during 20 weeks and returned at baseline levels at the end of the study (different treatment: $p = 0.049$). However, after 42 weeks, hs-CRP decreased by 36% and 38% in the group I and the group II (time 0–42 weeks: $p = 0.001$), and by 48% in the group III compared to baseline values, despite that hs-CRP in the group III was statistically lower than in other groups at baseline (different treatment: $p = 0.048$).

Discussion

Our dietary intervention demonstrated that overweight and obese female, consuming different CR diets had the similar body weight reduction by 5% after 4 weeks of the treatment and improved cardiometabolic risk factors. Generally, different CR diet groups achieved 12% (~11 kg) weight loss at 42 weeks of the treatment from initial body weight. When we analyzed WC and percentage of body fat as outcome variables, there were no differences in the effectiveness among the different CR weight-loss methods. Participants in different CR (20%, 50%, 70%/30%) groups during long-term protocol (42 weeks) with overall weight lost by 12%, had similar reduction in WC (11–12cm) and % of body fat (15–16%). These findings indicate that different CR diets have the same effect in reducing body weight and fat mass in overweight and obese women. Our results are consistent with the evidence that most short-term dietary interventions (8–24 weeks) produce body weight decreases of 3–10%, while longer-term protocols (25–52 weeks) produce weight reductions of 10–15% [6–8, 12–14, 19–20].

It is well known that dietary interventions are effective for weight loss [7, 21]. In overweight subjects when BMI is 25–29.9 kg/m^2, advice is to introduce CR, physical activity and lifestyle modification. Tokunaga and Furubayashi [22] suggested that the diets containing 1000–1200 kcal/day should be selected for obese patients. There was some evidence that CR diets lead to clinically significant weight loss regardless of which macronutrients stand out [23]. In this research, our participants had different CR diets (20%, 50% and 70%/30% CR), but with the same macronutrient content: carbohydrate (55–60% of total calories) proteins (15–20% of total calories) and fat (less than 30% of energy intake). Very LCDs with 600 kcal/day should not be used routinely for weight-loss therapy because it requires special monitoring and supplementation. So, in the diet group III we combined alternating daily CR; on the first day very LCD of 70% and on the second day moderate LCD with 30% CR of baseline energy requirements.

No dose-response link between greater degree of CR and larger amounts of weight loss was presented in scientific works. For example, 6% reduction from baseline values during 12–13 weeks of the procedure was shown with 16% and 23% CR diets [24, 25]. Our results are consistent with the statement that a degree of CR is not a crucial factor which affects the outcome of weight loss. There was some evidence that short-term diet (4–12 weeks) made significant reductions in body fat of 10–20%. Moderate-term trials (13–24 weeks) showed similar result, 11–34% in reduction of body fat from baseline [24–28].

Visceral obesity is closely related with greater risk of cardiovascular disease (CVD) and type 2 diabetes [29, 30]. For example, in the studies with 6–8% weight loss visceral fat mass was reduced by 6–13% from baseline [6, 30]. In our study with 12% weight loss (~11kg), WC was reduced by 11 cm (15% from baseline). These changes are important since WC is an important predictor for cardiometabolic risk and metabolic syndrome [6, 17].

Reductions in body weight are generally accompanied by favorable changes in metabolic disease risk parameters. It has been presented that loss of 5%–10% of the initial body weight is a sufficient to perform clinically evident improvement of the metabolic profile of obese persons [30–33]. Published studies suggest that, for instance, weight loss of 5–15% results in fairly consistent decreases in the serum of

Table 2

Cardiometabolic risk factors before and after 20 and 42 weeks of consummation of different calorie restrictive (CR) diets

Cardiometabolic risk factors	Group I – 20% CR (n = 37) $\bar{x} \pm SD$	Group II – 50% CR (n = 30) $\bar{x} \pm SD$	Group III – 70%/30% CR (n = 30) $\bar{x} \pm SD$	Different treatment [c] (probability)	Time interval 0–42weaks [d] (probability)	Effect Time x CR group [e] (probability)
Triglicerides (mmol/L)						
week 0	1.07 ± 0.46	1.21 ± 0.57	1.20 ± 0.98			
week 20	0.85 ± 0.96	1.08 ± 0.45	0.98 ± 0.76	$p = 0.893$ [c]	$p < 0.014$ [d]	$p = 0.727$ [e]
week 42	0.82 ± 0.40	0.87 ± 0.45	0.82 ± 0.38			
Change 0–20	-0.22 ± 0.40	-0.13 ± 0.59	-0.22 ± 0.65			
Change 0–42	-0.25 ± 0.53	-0.34 ± 0.67	-0.38 ± 0.80			
Total cholesterol (mmol/L)						
week 0	5.05 ± 1.04	5.03 ± 0.92	5.13 ± 1.06			
week 20	4.68 ± 1.02	4.95 ± 1.00	4.84 ± 1.02*	$p = 0.664$ [c]	$p < 0.001$ [d]	$p = 0.025$ [e]
week 45	4.70 ± 0.6	5.00 ± 1.05*	4.70 ± 0.95			
Change 0–20	-0.37 ± 0.51	-0.07 ± 0.40[b]	-0.27 ± 0.36			
Change 0–42	-0.35 ± 0.50	-0.35 ± 0.55[b]	-0.43 ± 0.51			
LDL cholesterol (mmol/L)						
week 0	3.35 ± 0.81	3.31 ± 0.86	3.24 ± 0.86			
week 20	2.93 ± 0.89	3.30 ± 1.01	3.03 ±0.85	$p = 0.412$ [c]	$p < 0.02$ [d]	$p = 0.024$ [e]
week 42	2.87 ± 0.87	3.35 ± 1.00[b]	2.82 ± 0.77			
Change 0–20	-0.41 ± 0.64	-0.01 ± 0.40[b]	-0.21 ± 0.36			
Change 0–42	-0.47 ± 0.51	0.04 ± 0.67[b]	-0.42 ± 0.67			
HDL cholesterol (mmol/L)						
week 0	1.46 ± 0.23	1.44 ± 0.26	1.34 ± 0.29			
week 20	1.50 ± 0.31[a]	1.53 ± 0.33[a]	1.29 ± 0.26	$p = 0.049$ [c]	$p = 0.654$ [d]	$p = 0.482$ [e]
week 45	1.52 ± 0.25	1.47 ± 0.31	1.34 ± 0.28			
Change 0–20	0.04 ± 0.31	0.04 ± 0.31	0.04 ± 0.31			
Change 0–42	0.07 ± 0.47[a]	0.03 ± 0.48	0.00 ± 0.29			
TG/HDL-cholesterol ratio						
week 0	0.79 ± 0.34	0.87 ± 0.45	0.92 ± 0.74			
week 20	0.66 ± 0.35	0.76 ± 0.44	0.76 ± 0.29	$p = 0.431$ [c]	$p < 0.001$ [d]	$p = 810$ [e]
week 45	0.58 ± 0.32	0.62 ± 0.38	0.60 ± 0.34			
Change 0–20	-0.13 ± 0.24	-0.13 ± 0.24	-0.13 ± 0.24			
Change 0–42	-0.22 ± 0.23	-0.25 ± 0.39	-0.32 ± 0.28			
Glucose (mmol/L)						
week 0	4.91± 0.44*	4.67 ± 0.65	4.57 ± 0.65			
week 20	4.82 ± 0.46	4.69 ±0.62	4.59 ± 0.33	$p = 0.012$ [c]	$p = 0.321$ [d]	$p = 0.113$ [e]
week 45	4.79 ± 0.45	4.50 ± 0.44	4.48 ± 0.34			
Change 0–20	-0.08 ± 0.45[a]	-0.08 ± 0.45[a]	-0.08 ± 0.45[a]			
Change 0–42	-0.12 ± 0.55	-0.17 ± 0.73[a]	-0.09 ± 0.55			
$HbA1_C$ (%)						
week 0	5.53 ±0.41	5.68 ±0.48	5.41 ± 0.27			
week 20	5.33 ± 0.34	5.36 ±0.90	5.31 ± 0.28	$p = 0.267$ [c]	$p < 0.001$ [d]	$p = 0.2.38$ [e]
week 42	5.27 ± 0.29	5.22 ± 0.54	5.06 ± 0.51			
Change 0–20	-0.19 ± 0.24	-0.19 ± 0.24	-0.19 ± 0.24			
Change 0–42	-0.25 ± 0.89	-0.45 ± 0.82[a]	-0.35 ± 0..8			
hs-CRP (mg/L)						
week 0	6.23 ± 2.25**	5. 75 ± 3.50**	4.75 ± 1.86			
week 20	4.88 ± 3.01[a]	4.30 ± 3.33	3.67 ± 1.91*	$p = 0.048$ [c]	$p < 0.001$ [d]	$p = 0.113$ [e]
week 42	4.01 ± 2.11	3.64 ± 2.19	2.46 ± 1.05*			
Change 0–20	-1.35 ± 2.61[a]	-1.35 ± 2.61[a]	-1.35 ± 2.61[a]			
Change 0–42	-2.22 ± 2.45	-2.11± 2.83	-2.29 ± 3.15			

Abbreviations: * $p < 0.05$, ** $p < 0.05$ [significantly different to the group III – 30%/70% CR (univariate ANOVA)].

[a] $p < 0.05$ [b] $p < 0.01$ [comparison of baseline characteristics at week 0 (one way ANOVA)].

[c] Comparison of effects of different CR treatments (repeated ANOVA).

[d] Comparison of changes over time among different CR groups [from weeks 0, to week 20 and 42 (repeated ANOVA)].

[e] Comparison of treatment effect among different CR groups [for the changes from week 0, to week 4, 8, 20 and 42 (repeated ANOVA)].

LDL – low density lipoprotein; HDL – low density lipoprotein; TG – triglycerides; HbA1 – glycosylated hemoglobin; hs-CRP – high sensivity C-reactive protein.

total cholesterol (5–20%), LDL cholesterol (5–20%), fasting glucose (5–10%), and insulin (10–40%) levels [6–9, 12–14, 16, 17, 19].

In our study, reductions in cardiometabolic risk factors were noticed in all CR diet groups. Less decrease of triglycerides levels (23%) was in the diet group I (20% CR) compared with another two groups, the group II (50% CR) and the group III (70%/30% CR) (28% and 31%, respectively). In our study, 50% CR intake had no effect on total and LDL-cholesterol serum levels in overweight and obese females regardless of the decrease of 12% in body weight. There was some evidence that plasma lipid concentrations (total cholesterol, LDL cholesterol and TG) had a tendency to decline after loss of 5% of body weight [34–36].

Previous studies in overweight and obese patients with diabetes type 2, indicated that modest weight loss of 5–10% from initial body weight markedly improve glycemic control and CVD risk factors after one year [37, 38]. In our study in overweight and obese non diabetic females with 12% reductions of initial weight during 42 weeks, different CR diet decreased fasting glucose levels (2–10%) and HbA1c (5–8%) from baseline levels with greater reduction in glucose levels (10%) in the group I (moderate 20% CR diet), although, $HbA1_c$% had better response with 50% CR (the group II) (change -0.45 ± 0.82%) compared to the 20% CR diet group (change -0.25 ± 0.89%). This is a worse response in non-diabetic obese or overweight females compared to evidence that in overweight or obese subjects with diabetes type 2 HbA1c has a tendency to decline (0.4%) after body weight loss of 5% [39]. Participants in the group III with combined alternating daily 70%/30% CR diet had low response to glucose levels reduction (only 2%).

However, no statistically significantly different changes in triglyceride and HDL-cholesterol concentrations, glucose levels and $HbA1_c$ and TG/HDL ratio among different CR diet groups were observed following long-term protocols of 42 weeks in our study. Interestingly, data from the Diet, Obesity, and Genes (DiOGenes) Study, first demonstrated that LCDs induced 8% weight loss in obese adults during treatment period of 8 weeks. Besides, this study showed that consuming one of the four diets with no caloric restriction, different in protein content or food glycemic index during 26 weeks, had similar effects on the serum lipid status and fasting glucose levels [39]. Some studies have shown that macronutrient composition of CR can change the blood lipid profile response to weight loss [40–42]. Recent papers have suggested that atherogenic index (TG/HDL-cholesterol ratio) is most useful for the discrimination of insulin resistance in individuals [43–45]. Our study showed that 12% reduction in body composition can induce decline (28–35%) in this biomarker of the disease risk in overweight and obese females.

CRP is an sensitive inflammatory marker [45, 46], whose values fall in the blood after loss of 10% of initial body weight [47]. Marked weight loss has also been found to reduce the concentration of hs-CRP [48, 49]. Significant reduction in body weight decreases the concentration of hs-CRP. In our study, 12% reduction in body weight produced potent reduction (35–48%) in hs-CRP levels. In a recent meta-analysis of 33 weight loss intervention studies, conclusion was that there was a strong correlation between declines in body weight and the hs–CRP levels. Also, this study supported possibility to reduce the hs–CRP levels by non-pharmacological method such as an ordinary weight reduction [50]. It is interesting that despite lower basal levels of hs-CRP in the diet group III compared to another two CR diet groups alternating daily CR of 70% and 30% showed higher reduction of hs-CRP (48% from baseline) compared with 20% and 50% CR diets. These effects are essential for reduction in the CVD risk because hs-CRP is CVD predictor and an inflammatory molecule accumulated in the atherosclerotic process [46]. DiOGenes study showed that hs–CRP could be, even more reduced by a diet with a low glycemic index and significantly less content of proteins [41].

This study has limitation because we did not divide our study population into two subgroups (overweight and obese women) and compare the effects of different CR diets between them. The reason for this is high dropout rate (60%) that may limit the use of the study results in public health programs.

Conclusion

Different caloric restriction diets with the same macronutrient content have the same effect in decreasing body weight and body fat, as well as similar effect in reduction of cardiometabolic risk factors in overweight and obese females. In addition, this study confirmed that reduced body weight have beneficial effect on reducing level of pro-inflamatory hs-CRP.

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Impulse oscillometry in evaluation bronchial hyperresponsiveness in patients with persistent allergic rhinitis

Impulsna oscilometrija u proceni bronhijalne hipereaktivnosti kod bolesnika sa perzistentnim alergijskim rinitisom

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Abstract

Background/Aim. Impulse oscillometry (IOS) is a method for estimating lung function which is used for early detection of bronchial hyperresponsiveness (BHR) and asthma. The aim of the study was to determine the prevalence of BHR, the correlation between spirometry and IOS and sensitivity and specificity of IOS in proving BHR in patients with persistent allergic rhinitis. **Methods.** The study included 81 patients with allergic rhinitis. From all of them, medical history was taken, allergy testing was done, as well as measurements of parameters of lung function by the IOS and spirometry before and after nonspecific bronchial provocation test with histamin via Aerosol provocative system. Changes of the IOS parameters to fall in FEV1 of 20% were measured and compared with changes in the spirometry parameters. After bronchial challenge test subjects were divided into two groups: the group with BHR (group 1) and that without BHR (group 2). **Results.** The mean age of participants was 25.7 ± 5.7 years, and 50.5% were men. Out of the total number of subjects with allergy rhinitis, 56 (58.9%) had a positive BPT. After bronchoprovocation an average increase in the group 1 was 88.15% for Rrs5, 111.98% for Fres, and for AX 819.69%. The high degree of correlation between the IOS and spirometry was proven in the group 2, while the whole group 1 had a weak correlation between parameters of these two methods. High sensitivity and low specificity for Rrs5 and Fres compared to FEV1 in diagnosing BHR was proven. **Conclusion.** The study demonstrated a high prevalence of BHR in the study group of patients with persistent allergic rhinitis, poor correlation in relation to the spirometric measurements in the group with BHR and a high sensitivity and low specificity of IOS for the detection of early changes in the airways.

Key words:
rhinitis, allergic; oscilometry; spirometry; respiratory function test; diagnosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Impulsna oscilometrija (IOS) je metod za procenu funkcije pluća koji se koristi za rano otkrivanje bronhijalne hiperreaktivnosti (BHR) i astme. Cilj rada je bio da se utvrde učestalost BHR, korelacija između IOS i spirometrije, senzitivnost i specifičnost IOS u dijagnozi BHR kod bolesnika sa perzistentnim alergijskim rinitisom. **Metode.** Od 81 ispitanika sa alergijskim rinitisom uzeta je anamneza, urađeno je alergološko testiranje, merenja funkcije pluća pomoću spirometrije i IOS pre i posle nespecifičnog bronhoprovokativnog testa (BPT) sa histaminom preko Aerosol provokacionog sistema. Merene su promene parametara IOS do pada FEV1 za 20% koje su upoređivane sa promenama spirometrijskih parametara. Nakon BPT ispitanici su bili podeljeni u grupu sa BHR (grupa 1) i bez BHR (grupa 2). **Rezultati.** Prosečno životno doba ispitanika iznosilo je 25,7 ± 5,7 godina, a 50,5% su bili muškaraci. Od ukupnog broja ispitanika sa alergijskim rinitisom 56 (58,9%) su imali pozitivan BPT. Posle bronhoprovokacije u grupi 1 prosečno povećanje za Rrs5 bilo je 88,15%, za Fres 111,98%, za AX 819,69%. Visok stepen korelacije dokazan je između IOS i spirometrije u grupi 2, dok je u grupi 1 utvrđena slaba korelacija između vrednosti parametara ova dva metoda. Dokazana je visoka senzitivnost i niska specifičnost za vrednosti Rrs5 i Fres u odnosu na FEV1 u dijagnostikovanju BHR. **Zaključak.** Dokazana je visoka prevalencija BHR u grupi pacijenata sa perzistentnim alergijskim rinitisom, loša korelacija u odnosu na spirometrijska merenja u grupi sa BHR i visoka osetljivost i niska specifičnost IOS u detekciji ranih promena u disajnim putevima.

Ključne reči:
rinitis, alergijski; oscilometrija; spirometrija; respiratorna funkcija, testovi; dijagnoza; osetljivost i specifičnost.

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Introduction

Allergic rhinitis (AR) is a global problem which occurs in 500 million people around the world. AR is a chronic inflammation of the mucous membranes of the nose that occurs after exposure to allergens that trigger inflammation of the nasal mucosa, which is mediated by immunoglobulin class E. AR symptoms are sneezing, obstruction in the nose, increased secretion from the nose, nasal itching and often hyposmia [1].

The guidelines Allergy Rhinitis and Its Impact on Asthma (ARIA) which define and propose standard diagnostic and therapeutic procedures are widely accepted in the world. According to the ARIA, AR is divided regarding the time of occurrence and duration of symptoms (intermittent and persistent) and regarding the severity (mild and moderate – severe), and is considered a major risk factor for asthma [1].

According to the latest guidelines of the Global Initiative of Asthma (GINA) from 2015 [2] asthma is defined as a heterogeneous disease (more phenotypes) which is usually characterized by chronic inflammation of the airways. The diagnosis of asthma is made on the basis of history data of respiratory symptoms, clinical findings, allergy tests and bronchodynamic tests for the detection of bronchial hyperresponsiveness (BHR) according to GINA guidelines. People suffering from asthma in 70–90% of cases are also suffering from AR [3]. Patients with AR have often expressed BHR without symptoms displayed, in other words, they have „asthma without asthma“ [4].

BHR was defined as a greater tendency to change the lumen of the airways in response to different substances or provocative stimuli. BHR is proving by bronchodynamic tests bronchodilator test (BDT) and bronchial challenge test (BCT) [2]. In patients with AR according to the recommendations of the ARIA there is an indication for bronchodynamic tests to prove any BHR [5].

AR and asthma are often associated disorders, covered by the term „one airway disease“. The concept of a single airway is based on the diagnostic and therapeutic implications [6, 7].

Impulse oscillometry [Impulse Oscillometry System (IOS)] is still non-standardized method of measuring lung function, which is used for determining the respiratory impedance over input sound pulses into the airway, and it is a result of interaction between the resistance (Rrs) and reactivity (Xrs) of the respiratory system [8]. Testing is conducted by impulse oscillometer and lasts only 20 to 30 sec and does not depend on the cooperation of the patient contrary standard methods for estimating pulmonary function, which is perfect in pulmonology and pediatric patients [9]. This method provides specific and additional information on standard measurements of lung function and is suitable for the detection of BHR [10].

The aim of the study was to determine the prevalence of BHR, a correlation between IOS and spirometry, and the sensitivity and specificity of respiratory impedance method compared to the conventional methods for estimating lung function in detection of BHR in patients with persistent AR.

Methods

Criteria used for selecting subjects for the study were as follows: patients of both sexes, non-smokers, aged 18–40 years, with the evidence of persistent type AR of and fulfilled ARIA criteria [1]. Criteria for excluding patients from the study were: contraindications to perform spirometry and bronhoprovocating test according to the recommendations of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) [11, 12], a contraindication for implementing tests for allergies according to the ARIA guidelines [1] and non-cooperation.

After BCT, subjects were divided into the group with BHR (group 1) and the one without BHR (group 2). The expected size of the sample was approximately 80 patients (± 10%).

This study was approved by the Ethics Committee of the Military Medical Center in Novi Sad, Serbia. All participants received instruction for BCT preparation, which contained a list of medicines and foods that should not be taken before testing (no taking short-acting beta agonists for 6 hours, long acting beta agonists for 12 hours, anticholinergics for 12 hours before the test, to avoid coffee, carbonated juices, tea and chocolate). The subjects also received instructions not to take antihistamines and corticosteroids that could modify the clinical picture of persistent AR and influence results of the prick test. The test was carried out in the following order: first, in all patients the diagnosis of persistent AR was on or off according to the ARIA guidelines [1] on the basis of a patient's history and alergology skin test. On the basis of the history data on the duration of symptoms, it was determined whether the patients with AR had intermittent or persistent type of the disease and, according to the intensity of the symptoms, whether they had mild od moderate – severe form of the disease [1].

The diagnosis of AR according to the ARIA guidelines [1] was based on a history of symptoms of AR and results of epicutaneous prick test with standard inhalant allergens [13]. The prick test was done with standard set of 10 inhalation allergens with saline solution as negative control and with 0.5% solution of histamine as positive control. Allergic hypersensitivity to certain allergens was considered positive if the papules at the site of allergen application were 3 or more millimeters in diameter.

Basic measurements of spirometry and IOS parameters were performed in both groups. The IOS was performed as recommended by the constructor (Impulse oscilometer series Master Screen IOS Care Fusion, Jaeger, Wurtzburg, Germany) [8]. Following parameters of the IOS were measured: total impedance at 5 Hz – Zrs5 (kP/L/s), total resistance at 5 Hz – Rrs5 (kP/L/s), resistance at 20 Hz – Rrs20 (kP/L/s), differential resistance at 5 and 20 Hz – Rrs5-Rrs20 (kP/L/s), a reactance at 5 Hz – Xrs5 (kP/L/s), Δ Xrs5 (Xrs5pred – Xrs5act); resonant frequency (Fres) (L/s), asthma index or Goldman index – AX (L/s). The results measured by the IOS were compared with the norms of the constructor [8].

Spirometry was performed on a series spirometer (Master Screen IOS, Care Fusion, Jaeger, Wurtzburg, Germany) by the standards of the ATS and ERS [14, 15], and following parameters were determined: forced vital

capacity (FVC) [1], forced expiratory volume in one second (FEV1) [1], the ratio of FEV1/FVC (%); forced expiratory flow at 50% of expiratory flow – (FEF 50) [1]; the ratio of forced expiratory flow at 25% and 75% of expiratory flow – [FEF 25/75 (%)]. The results of spirometric measurements were compared with the norms of the European Coal and Steel Community (ECSC) [14, 15].

Nonspecific BPT with histamine was performed with spirometry and the IOS through the Aerosol Provocation System (APS) (Care Fusion, Jaeger, Wurtzburg, Germany). Aerosol provocation system has a compressor with a flow rate of 7 L/min and working pressure of 0.9 bar. The system supports powerful jet nebulizer that produces aerosol. The nebulizers used for APS is a "DeVilbiss 646", and has particle diameter of 4.5 μm, and strength of 1,400 mg/min. APS has software which has two central parts, the test sequence and the observation module. Nonspecific BPT with histamine was done under the guidelines of the ATS and the ERS. Provocation test was performed by the first executed basic measurements of pulmonary function following with measurements after inhalation of bronchoprovocative substances. The first step included inhalation of l mL of physiological saline (NaCl 0.9%) and then the test was continued using the same model of histamine inhalation solution in increasing concentrations of 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.0 mg/mL. Stock solution was prepared in concentrations of 32 mg/mL and 4 mg/mL, at the Institute of Pharmacy, Military Medical Academy, Belgrade, using a software which automatically assigns the set of increasing concentrations from 0.03 to 2.0 mg/mL [16]. Nonspecific BPT with histamine ended when increasing concentrations of histamine achieved the final step of 2 mg/mL histamine or when the cumulative histamine concentration reached 3.8–4.0 mg/mL [17, 18]. The test was interrupted and was considered positive if the spirometric parameter FEV1 had decreased by 20% („cut off") referring to the basic values, and the IOS was positive („cut off") if there was an increase in Rrs5 by 40% or Fres by 35%. Exact consumption of histamine which led to decrease of FEV1 by 20% (PC20) was calculated on a straight line formula. Reports for BPT were originally designed to monitor all the analyzed parameters [19].

All statistical calculations were done by using commercial statistical software Statistica 7.0 StatSoft. Descriptive statistics included conventional parameters for assessing the data of central tendency (mean and median), as well as parameters for evaluation of group variability [standard deviation (SD), range, 95% confidence limits]. The distribution of some characteristics was presented as frequencies. The difference between the distribution characteristics of the group was checked using the χ^2 test. For comparison of lung function parameters between groups, Student's *t*-test was used. To assess the strength of the relationship between various parameters, the Pearson's coefficient of correlation was used. A statistically significant difference was considered in the case of $p < 0.05$, moderately significant in the case of $p < 0.01$ and highly statistically significant if $p < 0.001$.

Results

The study included 81 respondents subjected to nonspecific BCT with histamine. Out of the total number of respondents, 50.5% were men, and their average age was 5.7 ± 5.7 years (Table 1). Allergy testing showed that 55.1% of respondents were positive to mixture of allergens, 25.1% were positive to indoor allergens and 19.7% had pollen allergy. Out of the total number of subjects with AR, 56 (58.9%) had a positive BPT and proven bronchial hyperreactivity, while 25 (41.1%) had a negative BCT. Average consumption of histamine in the group with proven BHR was 1.530 mg/mL (Table 1), whereas in the group with negative BPT it was 3.949 mg/mL on average.

Table 1

Patients (n = 81) characteristics and basic measurements of lung function

Variable	$\bar{x}$ ± SD
Gender, male/female (%)	50.5 ± 49.5
Age, years	25.7 ± 5.7
PC20 mg/mL	1.530 ± 1.21
Zrs5 (%)	117.31 ± 40.17
Rrs5 (%)	112.04 ± 39.13
Rrs20 (%)	111.53 ± 4.09
Rrs5-Rrs20a	0.050 ± 0.042
Xrs5 - kPa/(L/s)	-0.10 ± 0.03
Fres - kPa/(L/s)	10.94 ± 3.00
AX - kPa/(L/s)	0.25 ± 0.16
FVC (%)	106.18 ± 12.16
FEV1 (%)	105.71 ± 15.45
FEV1/FVC (%)	86.21 ± 5.92
FEF50 (%)	95.95 ± 19.26
FEF25/75 (%)	98.13 ± 22.02

PC20 – A 20% reduction in the forced expiratory volume in one second (FEV1); Zrs – total respiratory impedence; Rrs – respiratory system resistance; Xrs – respiratory system reactance; Fres – resonant frequency; AX – asthma index; FVC – forced vital capacity; FEF – forced expiratory flow; $\bar{x}$ – mean value; SD – standard deviation.

Table 2 presents basic measurement values of the spirometry and the IOS paremeters in patients with (the group 1) and without BHR (the group 2). The differences between the value of the spirometry and IOS parameters values in the groups 1 and 2 were not statistically significant except those of FEF50 and FEF25/75.

Table 3 shows the mean values and standard deviations of differences between values of all the spirometry and IOS paramaters monitored in the groups 1 and 2 and determined before and after BCT. It was noted that the mean values of changes in the group 1 were even two times higher than those in the group 2. Table 4 shows the correlations between parameters measured before and after provocation test performed in the group 1 and 2. There was a significant correlation within the group 1 between the value of PC20 and ΔFEV1 (r = 0.41) (Table 5).

Table 2

Lung function in allergic rhinitis (AR) patients with and without bronchial hyperresponsiveness (BHR) – basic measurement

Variable	Group 1 (n = 56)	Group 2 (n = 25)	*p*
	x̄ ± SD	x̄ ± SD	
Zrs5 (%)	121.53 ± 46.46	107.87 ± 17.23	0.158671
Rrs5 (%)	116.04 ± 45.26	103.08 ± 17.01	0.170183
Rrs20 (%)	115.44 ± 39.09	102.77 ± 16.01	0.122977
Rrs5-Rrs20a	0.052 ± 0.044	0.046 ± 0.037	0.586672
Xrs5	-0.10 ± 0.03	-0.10 ± 0.02	0.974030
Fres	11.04 ± 3.14	10.70 ± 2.72	0.638597
AX	0.26 ± 0.16	0.24 ± 0.15	0.725618
FVC (%)	104.86 ± 11.54	109.14 ± 13.22	0.122134
FEV1 (%)	105.58 ± 10.64	106.02 ± 23.15	0.907481
FEV1/FVC (%)	86.26 ± 5.71	86.08 ± 6.48	0.898740
FEF50 (%)	92.61 ± 17.56	103.06 ± 21.30	0.025397*
FEF25/75 (%)	94.81 ± 20.89	105.56 ± 23.07	0.041658*

***p* < 0.05 is statistically significant. Group 1 – patients with BHR; Group 2 – patients without BHR.**
For other abbreviations see under the Table 1.

Table 3

Changes of spirometry and impulse oscilometry (IOS) parameters in the group with and without bronchial hyperresponsiveness (BHR)

Changes (%)	AR with BHR (n = 56)	AR without BHR (n = 25)	*p*
	x̄ ± SD	x̄ ± SD	
ΔZrs5	94.51 ± 52.73	61.89 ± 45.04	0.008854
ΔRrs5	88.15 ± 45.92	59.98 ± 41.14	0.010255
ΔRrs20	43.12 ± 30.50	32.11 ± 29.41	0.133096
ΔRrs5-R20	45.29 ± 29.60	27.87 ± 29.85	0.016929
ΔXrs5	141.51 ± 119.67	88.63 ± 96.55	0.055577
ΔFres	111.98 ± 65.14	75.09 ± 52.17	0.014710
ΔAX	819.69 ± 734.43	424.87 ± 449.23	0.015130
ΔFVC	-13.34 ± 8.26	-2.86 ± 4.68	0.000000
ΔFEV1	-25.66 ± 6.44	-8.41 ± 6.26	0.000000
ΔFEF50	-44.00 ± 8.91	-23.24 ± 14.70	0.000000
ΔFEF25/75	-44.4571 ± 9.86	-20.2140 ± 19.15	0.000000

For other abbreviations see under the Table 1.
AR – allergic rhinitis patients.

Table 4

Correlation between basic measurements of lung function and changes in FEV1 after BCT in the groups 1and 2

Measurement of	Group I (n = 56)				Group II (n = 25)			
FEV1	Rrs5	Xrs5	Fres	AX	Rrs5	Xrs5	Fres	AX
Basic	-0.02	0.27	-0.32	-0.35	-0.06	0.01	-0.24	-0.01
After BCT* (ΔFEV1)	-0.04	-0.07	-0.10	-0.16	-0.62	-0.51	-0.55	-0.55

*** BCT – Bronchial challenge testing; Group 1 – patients with bronchial hyperresponsiveness (BHR); Group 2 – patients without BHR.**
For other abbreviations see under the Table 1.

Table 5

Pearson's correlation coefficient between PC20 and parameters of the spirometry and IOS

Parameters	Pearson's correlation coefficient
ΔRrs5	0.14
ΔFres	0.10
ΔAX	0.06
ΔFEV1	0.41

IOS – impulse oscillometry.
For other abbreviations see under the Table 1.

Parameter Rrs5, as a marker of BHR in patients with AR showed a sensitivity of 82.14% and specificity of 36.0%. The positive and predictive values of Rrs5 were 74.19%, and 47.37%, respectively. Parameter Fres in proving BHR in patients with AR, showed sensitivity of 85.7% and specificity of 28%. The positive and negative predictive values of Fres were 72.73% and 46.67%, respectively (Table 6).

Table 6

Sensitivity and specificity of Rrs5/Fres in the bronchial challenge testing (BCT)

Parameters	ΔRrs5 (%)		ΔRrs5 (%)	
	Mean	(95% confidence interval)	Mean	(95% confidence interval)
Sensitivity	82.14	(72.16 – 92.12)	85.71	(76.60 – 94.83)
Specificity	36.00	(17.28 – 54.72)	28.00	(10.49 – 45.51)
Total accuracy	67.90	(57.79 – 78.02)	67.90	(57.79 – 78.02)
Positive predictive values	74.19	(63.36 – 85.03)	72.73	(62.04 – 83.42)
Negative predictive values	47.37	(25.03 – 69.71)	46.67	(21.55 – 71.79)

Rrs5/Fres – respiratory system resistance/resonant frequency.

Discussion

AR represents a prime risk factor for asthma, according to the ARIA guidelines [1, 7, 20]. Particular attention is paid to patients with AR and confirmed BHR, but without cardinal symptoms indicating asthma.

In everyday clinical practice the only method that reached the value of spirometry, a „gold standard“ in diagnostics of respiratory pathology, is the IOS [8].

In the scientific literature in Serbia, and some other countries, there are a few papers dealing with estimation of BHR by the IOS in patients with persistent RA [21, 22].

In our study nonspecific BPT with histamine revealed that 58.9% patients with AR had asymptomatic BHR (the group 1). Among patients who reacted to the provocative concentrations between 1 and 16 mg/mL of histamine that led to a drop in FEV1 of 20% (PC20), but have no symptoms of asthma, there were several subgroups: 1) patients with mild intermittent asthma who felt no bad asthma symptoms; 2) patients who did not experience the qualm in the chest as abnormal after exertion or provocation test; 3) patients who never had experience with effort or inhalation of harmful substances; 4) patients with mild degree of BHR, which was demonstrated after viral upper respiratory tract infections or smoking; 5) asymptomatic patients with asthma that would become clinically manifested in the future [15, 23]. Heppt et al. [24] reported that BHR was present in 10% to 50% of patients with AR. Zhong et al. [25] showed that in about 1.5% and 45% of asymptomatic people with AR and proven BHR asthma could develop in the future, in a period of 2–3 years. The group of Australian authors established BHR in 11.4% of random sample of the adult population in Australia [26]. Valdesoiro et al. [27] demonstrated that patients with AR without asthma symptoms and with confirmed BHR may have subclinical inflammation. They processed a total of 135 patients with AR, out of which BPT was positive in 24% respondents. Cuttitta et al. [28] investigated the prevalence of BHR in a sample of nonasthmatic children with AR by metacholin test; there were 31 (61%) children without, and 6 (20%) with evidence of BHR. Riccioni et al. [29] presented that 54.5% subjects with perennial AR, demonstrated BHR [29]. Gaur et al. [30] showed that inhaled allergens were predictors of BHR in 32 adult patients with AR and proved the presence of asymptomatic BHR in 81.2% of subjects [30]. Presented studies shows different frequency of BHR in subjects with AR.

Analysis of basic spirometric parameters showed that their mean values were far above the normal ones. In the paper of the Korean authors [31] there were seen similar mean values of basic spirometric parameters in the asthmatic patients.

Analysis of the basic IOS parameters showed similarity in the mean values between the groups 1 and 2. Mean values of the IOS parameters had a high degree of variability indicating broad range between the maximum and minimum values. This is one of the key factors why this method did not become a standardized test for estimating lung function. One of the studies that investigated the variability in the measurement of respiratory impedance were made by Goldman et al. [32] They showed a daily variability in the parameters Rrs5, Rrs5-Rrs15 (difference of resistance at 5 Hz and 15 Hz) and AX in patients with asthma. According to constructor's recommendation [8] for the threshold values for Rrs5 over 150% of the predicted values, Rrs20 over 150% of the predicted values and Xrs5 over -0.15 kPa/(l/s) (the difference between the active and predicted value) separate normal from abnormal findings. In addition to all of spirometry parameters which were far above the limits of normal values in the group 1, there were 5 patients with abnormal values of Rrs5 and 6 patients with abnormal values of Xrs5, which indicates that the IOS, unlike spirometry, can establish changes in the airways before bronchodynamic tests and indicate inflammation in the airway.

After testing the bronchial response to histamine patients were allocated to the groups of patients with and without BHR. The group with BHR had high mean values of the IOS parameters that were accompanied by high values of SD, which was especially pronounced for ΔAX. In the group 2, high mean values of the IOS parameters were also shown. There were no significant differences in values of the IOS and spirometry parameters between the two groups. Within the group with positive BHR there was no significant correlation between ΔFEV1 and parameters of the IOS, while in the group without evidence of BHR a significant correlation between ΔFEV1 and monitored parameters of the IOS was shown. Nonspecific BPT with histamine was positive with an average expenditure of the provocation substance of 1,530 mg/mL to achieve PC20, while in the group with negative test the mean value of the consumption of histamine was 3,949 mg/mL. Similar consumption of

histamine in provocation tests was shown in a study of Skiepko et al. [33].

The sensitivity and specificity of the IOS as a new method gives hope that in the future it can become the conventional method for estimating lung function and a great support to other spirometric methods. Based on the study of Marotta et al. [34] and the proposal of constructor Hans Jurgen Smith, we took „cut off" value for Rrs5 to demonstrate BHR [8]. In our study, sensitivity of the method for confirmation of BHR in patients with AR for Rrs5 was 82.14% and the specificity was 36.0%, and for Fres the sensitivity was 85.7% and the specificity 28%. The high sensitivity of both parameters indicates that you really can prove BHR in the respectable percentage of patients with AR. Schulze et al. [35] evaluated the spirometry and IOS during methacholine challenge test, and showed an increase of Rrs5 to 45.2% with a reduction in FEV1 of 20% in 70–80% of patients with BHR detected by the IOS. During BCT an increase in Rrs5 preceded the decrease in FEV1 of 20%. Komarow et al. [22] examined the sensitivity of the IOS in relation to the spirometry test in children of average age of 7 years with and without asthma and demonstrated that resistance at 5 Hz had a high sensitivity (0.73) and low specificity (0.34), and the reactance at 5 Hz, had a low sensitivity (0.59) and specificity (0.31) for Xrs5, which is similar to our results. Shin et al. [36] studied the bronchodilatory response among preschool children with asthma and healthy children, and demonstrated high sensitivity (0.92) and poor specificity (0.52) of Rrs5 in comparison with FEV1.

Conclusion

The study demonstrated a high prevalence of BHR in the group of patients with persistent AR, poor correlation with spirometric parameters in the group of patients with BHR, as well as high sensitivity and low specificity of IOS in detection of early changes in the airways.

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Neutrophil-to-lymphocyte ratio in pediatric acute appendicitis

Neutrofilno-limfocitni odnos u akutnom apendicitisu kod dece

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Abstract

Background/Aim. Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent abdominal operation in the pediatric surgery. Diagnosis of AA in children is more difficult due to a lack of cooperation and poor clinical history data, leading to significant number of misdiagnostic cases. Our aim was to explore whether neutrophil to lymphocyte ratio (NLR) may be useful in diagnosis and follow-up of AA in children. **Methods.** Prospective investigation of NLR values in 129 consecutive patients admitted to the Mother and Child Healthcare Institute of Serbia and referred for surgery due to AA was performed. According to the pathohistological findings, patients were divided into 3 groups: normal/early, uncomplicated (phlegmonous) and complicated (gangrenous or/and perforated) AA. Laboratory analysis was done preoperativly and on the 1st and the 3rd postoperative days. **Results.** Statistically significant differences of NLR values were found in the different time points in total of patients and per groups. Some statistically significant differences of NLR values among histopathological groups were recorded. Investigations of correlation of NLR and other laboratory and clinical parameters showed strong positive correlation between NLR and C-reactive protein postoperatively and between NLR and Pediatric Appendicitis Score (PAS) preoperatively. Strong negative correlation with preoperative symptoms duration (PSD) were also present. Optimal cutoff NLR value between negative and positive appendectomies was 6.14. **Conclusion.** NLR provides well monitoring of progression of AA in children and, its cutoff values may help in distinguishing the phases of AA. Because of that, NLR should be used in diagnosis of AA in children.

Key words:
appendicitis; neutrophils; lymphocytes; diagnosis; diagnosis, differential; prognosis; child.

Apstrakt

Uvod/Cilj. Akutni apendicitis (AA) predstavlja najčešće hitno stanje, a apendektomija najčešću abdominalnu operaciju u pedijatrijskoj hirurgiji. Dijagnoza AA kod dece je otežana nedostatkom saradnje prilikom pregleda i nepreciznim anamnestičkim podacima, što dovodi do većeg broja nepravilno dijagnostikovanih slučajeva. Cilj rada je bio da se ispita da li neutrofilno-limfocitni odnos (NLR) može biti koristan u dijagnostici i praćenju AA kod dece. **Metode.** Sprovedeno je prospektivno ispitivanje vrednosti NLR kod 129 bolesnika primljenih u Institut za zdravstvenu zaštitu majke i deteta Srbije, planiranih za hiruršku intervenciju zbog AA. Prema patohistološkom nalazu, bolesnici su podeljeni u tri grupe: normalni/rani, nekomplikovani (flegmonozni) i komplikovani (gangrenozni i/ili perforativni) AA. Laboratorijska ispitivanja su vršena preoperativno, kao i prvog i trećeg postoperativnog dana. **Rezultati.** Analizom je utvrđeno postojanje statistički značajnih razlika u vrednostima NLR preoperativno, prvog i trećeg postoperativnog dana kako na ukupnom uzorku tako i po formiranim grupama. Zabeležene su i statistički značajne razlike u vrednostima NLR između patohistoloških grupa. Ispitivanjem korelacija NLR sa drugim laboratorijskim i kliničkim parametrima, utvrđena je snažna korelacija sa C-reaktivnim proteinom postoperativno i snažna korelacija sa *Pediatric Appendicitis Score* (PAS) preoperativno. Takođe, preoperativno je utvrđena i snažna negativna korelacija sa preoperativnim trajanjem simptoma. Optimalna razgraničavajuća vrednost NLR za negativne i pozitivne apendektomije iznosila je 6.14. **Zaključak.** Parametar NLR omogućuje dobro praćenje progresije AA kod dece. Razgraničavajuće vrednosti NLR mogu biti od koristi u određivanju faza bolesti, zbog čega bi NLR trebalo koristiti u dijagnostici AA kod dece.

Ključne reči:
apendicitis; neutrofili; limfociti; dijagnoza; dijagnoza, diferencijalna; prognoza; deca.

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Introduction

Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent abdominal operation in the pediatric surgery [1]. Despite new diagnostic methods [scoring systems, ultrasound, computed tomography (CT), nuclear magnetic resonance (NMR)], AA remains the most misdiagnosed surgical cause of pediatric acute abdomen [2].

Negative appendectomy is an operation done for suspected appendicitis with normal appendix on histological evaluation. A certain rate of these negative explorations for suspected appendicitis is accepted as a good surgical practice because of the devastating impact of perforated appendicitis. Especially in girls, negative appendectomy appears with incidence of up to 30% of all suspected appendicitis [3].

Missed or delayed diagnosis of appendicitis increases the possibility of perforation, which has the highest incidence in small children [4] and results in a fivefold increase of complication rate in the postoperative period [5]. So, in the routine clinical practice it is important to find out whether AA is the cause of symptoms and if it is, to distinguish complicated (gangrenous and perforated) from uncomplicated AA.

Early (catarrhal) appendicitis is characterized by the presence of neutrophils in the lumen or focally in the mucosa, often with the absence of any clinical symptoms. The significance of this finding is controversial since it is often found in patients as an incidental finding [6].

Diagnosis of AA in children is more difficult due to a lack of cooperation and poor clinical history data. Routine laboratory parameters we used for diagnosis of AA in children are white blood cells (WBC) count with leukocyte formula and C-reactive protein (CRP). Neutrophil to lymphocyte ratio (NLR) is directly derived from the WBC, and its diagnostic relevance in AA was studied more in adults, although AA is more common in children. Expected differences in the results are based primarily on the diversity of immune mechanisms in children and adults.

In this prospective investigation we analyzed NLR during AA in children, before operation and during postoperative period. At the same time, other laboratory parametars, including CRP, were mesured and patients condition was estimated by Pediatric Appendicitis Score (PAS).

Methods

Patients admitted to the Mother and Child Healthcare Institute of Serbia in Belgrade and referred for surgery after established diagnosis of AA, were consented according to the good clinical practice (GCP). In total, 129 consecutive patients, 3 to 16 years old, were analyzed from May to November 2015. Children younger then 3 years were not recruited. Patients with other actual acute disease were excluded from the study as well as the patients with operative finding of some other abdominal inflammation. The study was approved by Ethics Committee of Mother and Child Healthcare Institute of Serbia.

After obtaining anamnesis data, including duration of symptoms, blood sampling was done for determining the WBC count and CRP, and PAS was calculated. Two additional blood samplings were performed at the 1st and at the 3rd postoperative day for the same laboratory analysis.

In the process of the WBC count determination, hematological autoanalyzer Advia 120/2120 (Simens) was used. Calculation of NLR was done automatically as the percentage of neutrophils divided by the percentage of lymphocytes, using the formula after entering data in Excel table.

For statistical analysis we used PRISM GraphPad softver version 5.01. Correlations (Spearman's rho) and Mann–Whitney U-test were calculated for comparative statistics (z-score and two-tailed P). The cutoff values were determined using the receiver operating characteristic (ROC) analysis. The optimal cutoff value was represented as the most prominent point on the ROC curve for sensitivity and specificity. A *p* value less then 0.05 was considered statistically significant.

Results

We analyzed 129 consecutive patients, 77 boys and 52 girls (male/female ratio 1,48), with average age of 10.43 ± 4.02 (boys: 10.03 ± 3.90; girls: 11.03 ± 4.16). Preoperative symptoms duration in days, in total of patients was 1.845 ± 1.61. Average preoperative pediatric PAS in total of patients was 7.31 ± 1.87, median 8 (2–10). Length of hospital stay in total of patients was 8.12 ± 4.09 days in average.

A total number of patients were separated into 3 groups. The first group represented patients with normal appendix and early stage of appendicitis as histopathological finding (NEAA); the second group, consisted of patients with phlegmonous or uncomplicated appendicitis (UAA); the third group were the patients with gangrenous and/or perforated appendicitis noticed as complicated appendicitis (CAA). Demographics and distribution of patients in the 3 formed groups are shown in the Table 1. Recorded preoperative parameters as well as those from the 1st and the 3rd postoperative days in the formed groups are listed in Table 2.

Table 1

Demographic characteristics and distributions of patients in the three formed groups

Group of patients	Patients' age (years)					
	(3–8)		(9–12)		(13–16)	
	male	female	male	female	male	female
NEAA (n = 23)	5	4	2	3	3	6
UAA (n = 50)	9	5	10	6	10	10
CAA (n = 56)	17	8	10	4	11	6
Total	31	17	22	13	24	22
	48		35		46	

NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis.

Table 2

Preoperative parameters and parameters from the 1st and the 3rd postoperative days in the formed grouops

Parameters	Group of patients		
	NEAA (n = 23)	UAA (n = 50)	CAA (n = 56)
PSD (days), $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	3.28 ± 2.66	1.24 ± 0.66	1.79 ± 1.26
PAS, $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	6.56 ± 2.02	6.76 ± 1.83	8.11 ± 1.56
WBC (10^9/L), $\bar{x} \pm SD$			
before surgery	12.31 ± 4.27	16.39 ± 5.82	19.31 ± 6.92
1st postop. day	11.62 ± 2.79	11.88 ± 3.44	13.39 ± 5.62
3rd postop. day	7.82 ± 2.04	7.76 ± 2.47	10.21 ± 6.52
Ne (%), $\bar{x} \pm SD$			
before surgery	69.42 ± 10.09	79.14 ± 12.33	83.26 ± 6.54
1st postop. day	77.59 ± 7.58	77.07 ± 7.61	78.74 ± 9.22
3rd postop. day	60.81 ± 6.14	59.35 ± 11.53	67.35 ± 12.74
Ly (%), $\bar{x} \pm SD$			
before surgery	20.34 ± 8.87	12.93 ± 9.54	8.49 ± 4.14
1st postop. day	14.49 ± 6.09	15.12 ± 5.89	12.83 ± 7.34
3rd postop. day	26.78 ± 5.95	27.96 ± 10.33	20.92 ± 10.60
NLR, $\bar{x} \pm SD$			
before surgery	4.79 ± 4.00	10.66 ± 8.13	14.10 ± 11.94
1st postop. day	6.73 ± 4.48	6.29 ± 3.56	8.61 ± 6.02
3rd postop. day	2.44 ± 0.86	2.65 ± 1.66	4.59 ± 3.32
CRP (mg/L), $\bar{x} \pm SD$			
before surgery	44.75 ± 48.9	61.88 ± 55.40	140.9 ± 90.83
1st postop. day	53.91 ± 42.58	61.88 ± 55.40	140.9 ± 90.83
3rd postop. day	37.88 ± 31.79	34.53 ± 32.53	89.14 ± 61.10
MPXI, $\bar{x} \pm SD$			
before surgery	-0.42 ± 5.46	-2.49 ± 5.92	-1.30 ± 5.57
1st postop. day	1.87 ± 3.34	-3.94 ± 6.95	-2.37 ± 7.07
3rd postop. day	0.21 ± 4.52	-5.66 ± 8.07	-3.43 ± 6.94
LOS (days), $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	8.91 ± 5.42	7.56 ± 3.38	8.27 ± 4.05

PSD – preoperative symptoms duration; PAS – Pediatric Appendicitis Score; WBC – white blood cell; Ne – neutrophils; Ly – lymphocytes; NLR – neutrophil/lymphocyte ratio; CRP – C–reactive protein; LOS – length of stay; MPXI – mieloperoxidase index; NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis.

NLR values in the different time points samples in total of patients and per groups

In total of 129 patients, statistically difference was recorded between values of NLR preoperatively, at the 1st and at the 3rd postoperative day (Figure 1A). NLR decreased from preoperative time point to the 3rd postoperative day, showing high statistical difference between preoperative and NLR value on the 1st day (11.10 ± 10.03 *vs.* 7.38 ± 5.01, $p = 0.0021$) and very high statistically significant difference between preoperative and NLR value that on the 3rd day (11.10 ± 10.03 *vs.* 3.42 ± 2.60, $p < 0.0001$) as well as between NLR values on the 1st and the 3rd postoperative day (7.38 ± 5.01 *vs.* 3.42 ± 2.60, $p < 0.0001$).

A statistically significant differences in NLR values among preoperative, the 1st, and the 3rd postoperative day samples were also registered within each individual histopathological group but with different pattern in the NEAA group. Namely, in the NEAA group, NLR values were highest on the 1st postoperative day, with statistically significant difference when compared with preoperative NLR values (6.73 ± 4.48 *vs.* 4.79 ± 4.00, $p = 0.013$), and very high statistically significant difference when compared with NLR values on the 3rd postoperative day (6.73 ± 4.48 *vs.* 2.44 ± 0.86, $p < 0.0001$). Preoperative NLR values were higher than NLR values on the 3rd postoperative day with high statistically significant difference (4.79 ± 4.00 vs. 2.44 ± 0.86, $p = 0.0074$, Figure 1B). In the UAA group, the

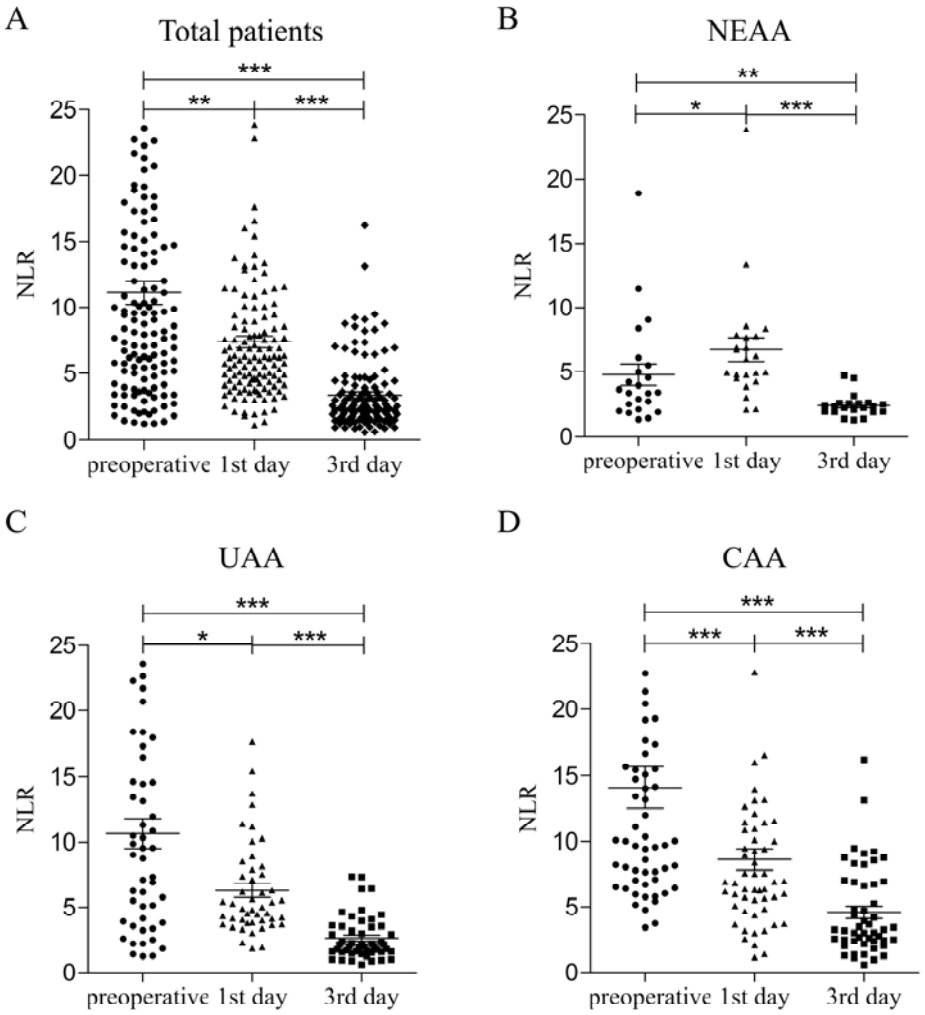

Fig. 1 – Comparison of the neutrophil to lymphocyte ratio (NLR) values measured in three different time points regarding to surgery: A) In total acute appendicitis (AA) patients, the highest NLR values were observed in the preoperative samples, with significant decrease toward the 3rd postoperative day; B) In the normal/early acute appendicitis (NEAA) group, the highest values were registered on the 1st postoperative day, with subsequent significant decrease toward the 3rd postoperative day; C) The NLR values in the uncomplicated acute appendicitis (UAA) and D) the complicated acute appendicitis (CAA) group showed similar pattern, being the highest in the preoperative samples with significant difference when compared with the 1st and the 3rd postoperative day samples. [mean ± standard error of mean (SEM), Mann-Withney test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)].

NLR values were highest in the preoperative samples, significantly higher when compared with samples on the 1st postoperative day (10.66 ± 8.13 *vs.* 6.29 ± 3.56, $p = 0.0148$), and higher than NLR values on the 3rd postoperative day with very highly statistically significant difference (10.66 ± 8.13 *vs.* 2.65 ± 1.70, $p < 0.0001$). The differences between preoperative NLR values and NLR values on the 3rd postoperative day were also very highly statistically significant in this group (6.29 ± 3.56 *vs.* 2.65 ± 1.70, $p < 0.0001$, Figure 1C). In the CAA group, again, NLR values were the highest in preoperative samples, with very high statistically significant difference when compared with NLR values on the 1st day postoperatively (14.10 ± 11.94 *vs.* 8.61 ± 6.02, $p = 0.0005$) and on the 3rd postoperative day (14.10 ± 11.94 *vs.* 4.59 ± 3.32, $p < 0.0001$). The NLR values the 1st postoperative day were higher than NLR values on the 3rd postoperative day with very high statistically significant difference (8.61 ± 6.02 vs. 4.59 ± 3.32, $p < 0.0001$, Figure 1D).

In both gender groups, statistically significant differences were also present among NLR values of the 3 blood sampling time points (Figure 2). In boys, significant difference was recorded between preoperative and NLR values on the 1st postoperative day (11.27 ± 9.57 vs. 7.53 ± 4.77, $p = 0.0101$), while the differences between preoperative and NLR values on the 3rd postoperative day as well as between

NLR values on the 1st and the 3rd postoperative day were very highly statistically significant (11.27 ± 9.57 vs. 3.44 ± 2.42, $p < 0.0001$; and 7.53 ± 4.77 vs. 3.44 ± 2.42, $p < 0.0001$, respectively, Figure 2A). In girls, very high statistically significant differences were found between NLR values on the preoperative time point and those on the 3rd postoperative day and between these values on the 1st and the 3rd postoperative day (10.86 ± 10.75 vs. 3.39 ± 2.87, $p < 0.0001$; and 7.17 ± 5.37 vs. 3.39 ± 2.87, $p < 0.0001$, respectively, Figure 2B).

NLR values between different histopathological groups in the 3 time points

In the preoperative samples, the lowest NLR values were found in the NEAA group, highly significantly lower in comparison with the UAA group (4.79 ± 4.00 vs. 10.66 ± 8.13, $p = 0.001$) and very highly significantly lower in comparison with the CAA group (4.79 ± 4.00 vs. 14.10 ± 11.94, $p < 0.0001$). Statistically significant difference was not reached in comparison with the preoperative NLR values between the UAA and CAA groups (Figure 3A). However, on the 1st postoperative day, the NLR values were statistically significant higher in the CAA group when compared with the UAA group (8.61 ± 6.02 *vs.* 6.29 ± 3.56, $p = 0.0175$, Figure 3B). Statistically significant difference in NLR values between the NEAA and UAA and between NEAA and CAA groups on the 1st postoperative day was not found. On the 3rd postoperative day, the highest NLR values were still in the CAA group, with statistically high significant difference when compared with the NEAA group (4.59 ± 3.32 vs. 2.44 ± 0.86, $p = 0.0036$) and with very high statistically significant difference in comparison with NLR values the UAA group (4.59 ± 3.32 vs. 2.65 ± 1.66, $p = 0.0006$). Statistically significant difference was not found in NLR values between the NEAA and UAA groups on the 3rd postoperative day (Figure 3C).

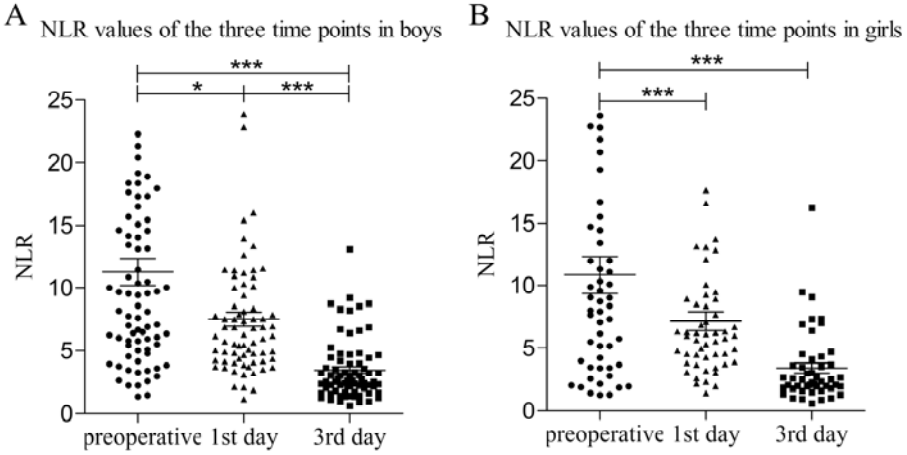

Fig. 2 – Comparison of the neutrophil to lymphocyte ratio (NLR) values measured in three different time points regarding to surgery; A) showing the highest values in the preoperative samples, with significant decrease toward the 3rd postoperative day in boys, as well as, in girls B) [(mean ± SEM, Mann-Withney test, *$p < 0.05$, ***$p < 0.0001$)].

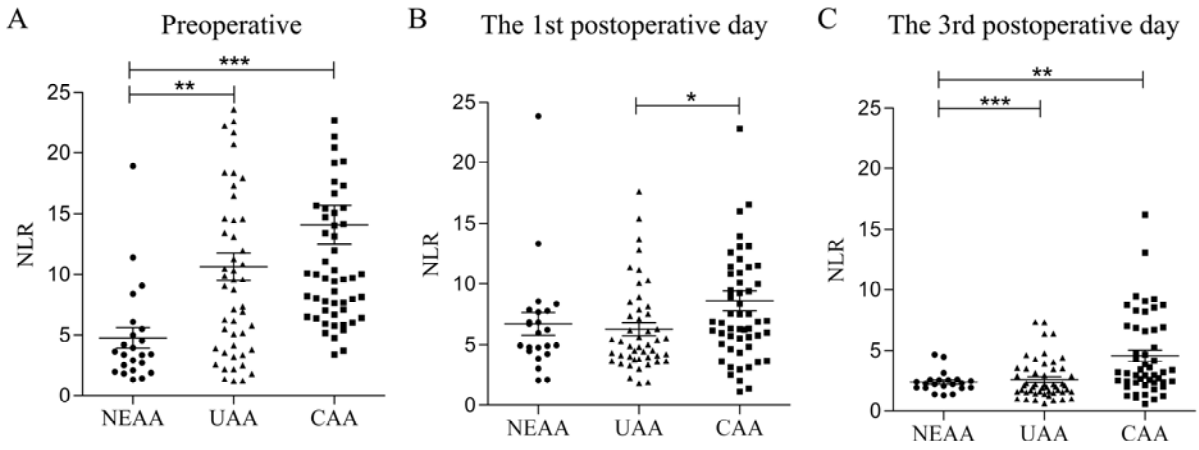

Fig. 3 – Differences in the neutrophil to lymphocyte (NLR) values between different histopathological groups at the three time points: A) showing the highest values in the CAA group in the preoperative samples, B) in the 1st day samples, and C) the 3rd day samples [mean ± standard error of mean (SEM), Mann-Withney test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)]. Explanation for NEAA, UAA and CAA see under Table 1.

NLR correlates inversely with PSD and positively with PAS and CRP

In total of 129 patients, the NLR values showed significant inverse correlation with the preoperative symptoms duration (PSD) measured in days (Spearman's r = -0.3190, p = 0.0002; Figure 4A). At the same time, duration of symptoms was significantly different among the different histopathological groups (not shown).

On the other hand, NLR showed a significant positive correlation with PAS in total AA patients (Spearman r = 0.4899, $p < 0.0001$; Figure 4B), as well as in the NEAA group, the UAA group, male and female groups, separately (not shown). Preoperatively, the mean PAS values were different between the NEAA and the UAA group as well as between the UAA and the CAA group (not shown).

In total AA patients, NLR showed a strong correlation with CRP on the 1st (Spearma'n r = 0.4082, $p < 0.0001$, Figure 5A) and the 3rd postoperative day (Spearman's r = 0.5814, $p < 0.0001$, Figure 5B). Statistically significant correlation between NLR and CRP was not recorded in the preoperative samples.

Within the histopathological groups significant correlation between NLR and CRP was found in the UAA group on the 3rd day (Spearman's r = 0.0336, p = 0.3175, Figure 6A), in the CAA group on the 1st postoperative day (Spearman's r = 0.6040, $p <$ 0.0001, Figure 6B) and again in the CAA group on the 3rd postoperative day (Spearman's r = 0.7063, $p < 0.0001$, Figure 6C).

By gender, correlation between NLR and CRP showed significant positive relationship on the 1st and the 3rd postoperative day in both, boys (Spearman's r = 0.3546, p = 0.0026; and Spearman's r = 0.6622, $p < 0.0001$, Figure 7A and 7B, respectively) and girls (Spearman r = 0.4743, p = 0.0006; and Spearman's r = 0.4463, $p < 0.0017$, Figure 7C and 7D, respectively). There was no significant correlation between NLR and CRP in the preoperative samples within both gender groups.

NLR did not show correlation with milloperoxidase index (MPXI) and length of hospital stay neither in the total sample nor by gender and histopathological groups.

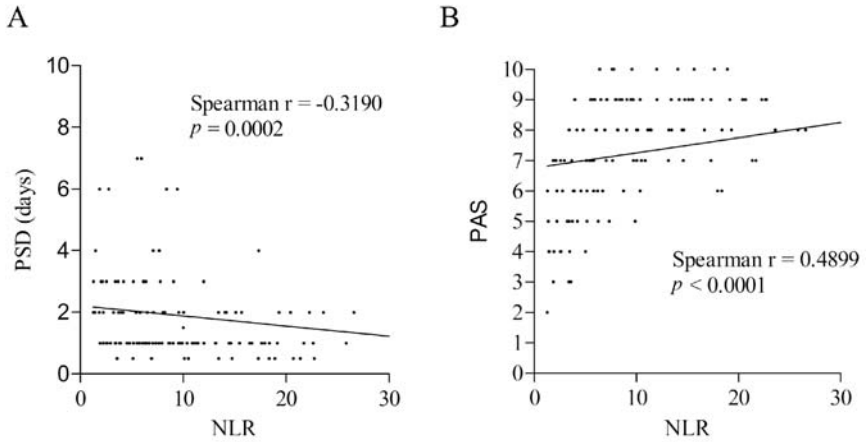

Fig. 4 – A) Correlation between neutrophil to lymphocyte ratio (NLR) and preoperative symptoms duration (PSD) in total acute appendicitis (AA) patients, showing significant inverse relationship; B) Correlation between NLR and pediatric appendicitis score (PAS) in total AA patients, showing significant positive relationship. (Spearman's correlation test).

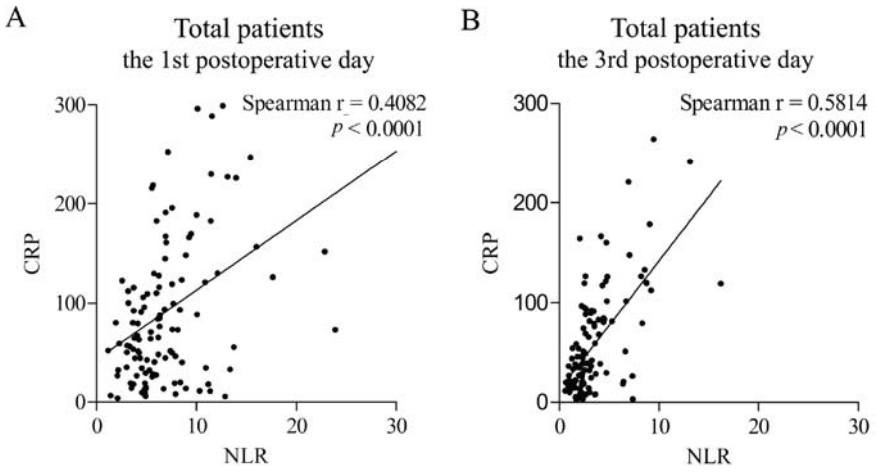

Fig. 5 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) in total acute appendicitis (AA) patients, showing significant positive relationship in: A) the samples taken on the 1st postoperative day, as well as in B) the samples taken on the 3rd postoperative day (Spearman's correlation test).

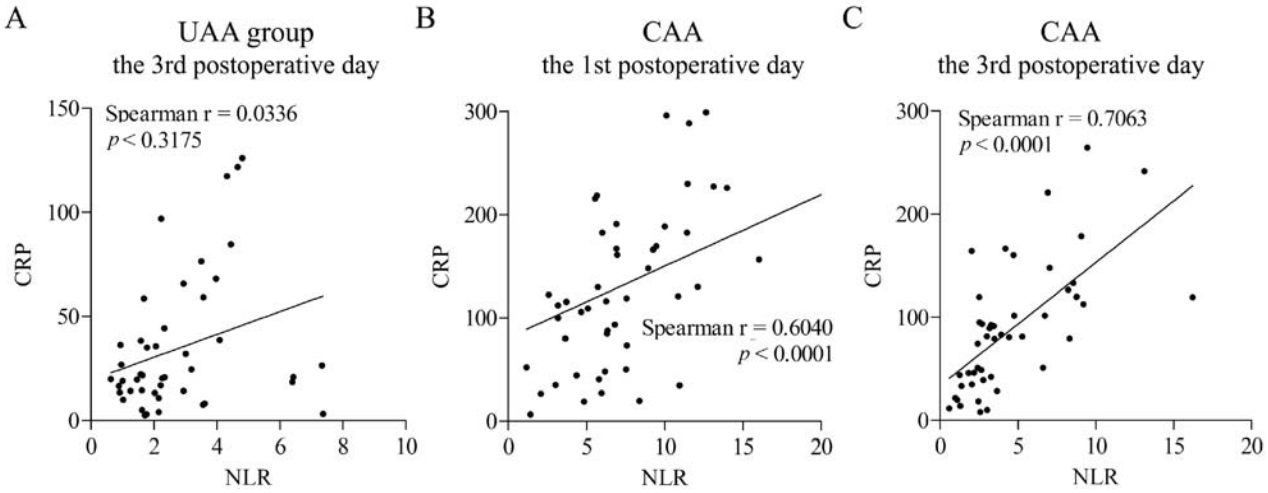

Fig. 6 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) within different histopathological groups, showing significant positive relationship in: A) the samples from the uncomplicated acute appendicitis (UAA) group taken on the 3rd postoperative day; B) the samples from the complicated acute appendicitis (CAA) group taken on the 1st postoperative day and C) the samples from the CAA group taken on the 3rd postoperative day (Spearman's correlation test).

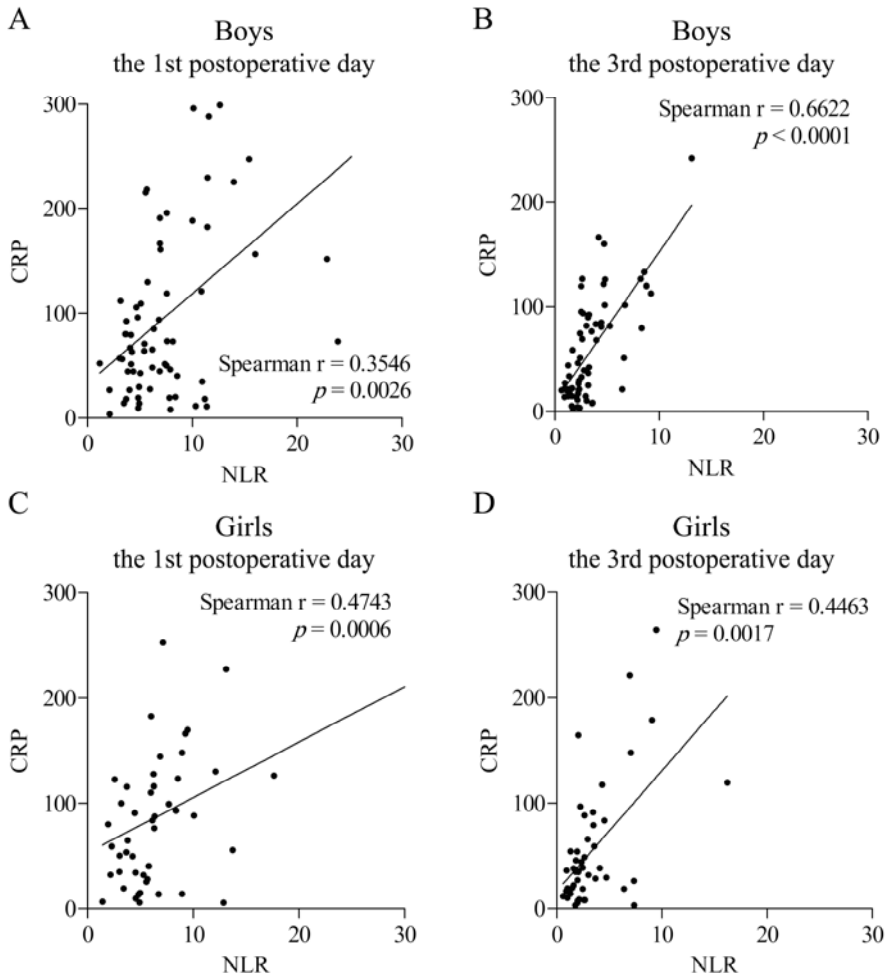

Fig. 7 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) within gender groups, showing significant positive relationship in boys: A) on the first, as well as B) on the third postoperative day. Correlation between NLR and CRP in girls, also showed significant positive relationship C) on the first, as well as D) on the third postoperative day (Spearman's correlation test).

NLR cutoff values among histopathological groups

Optimal cutoff NLR value between the NEAA and the UAA group was 5.06, with sensitivity of 71.43% and specificity of 73.91%, which represents test with moderate accuracy (AUC = 0.7409, p = 0.001011; Figure 8A). Between the UAA and the CAA group, optimal cutoff NLR value was 6.325, with sensitivity of 83.93% and specificity of 40.82%, which represents test with poor accuracy (AUC = 0.6023, p = 0.06986; Figure 8B). Optimal cutoff NLR value between the NEAA and the IAA (acute inflamed appendix; UAA + CAA) group was 6.14, with sensitivity of 74.53% and specificity of 82.61%, which represents test with good accuracy (AUC = 0.8175, $p < 0.0001$; Figure 8C).

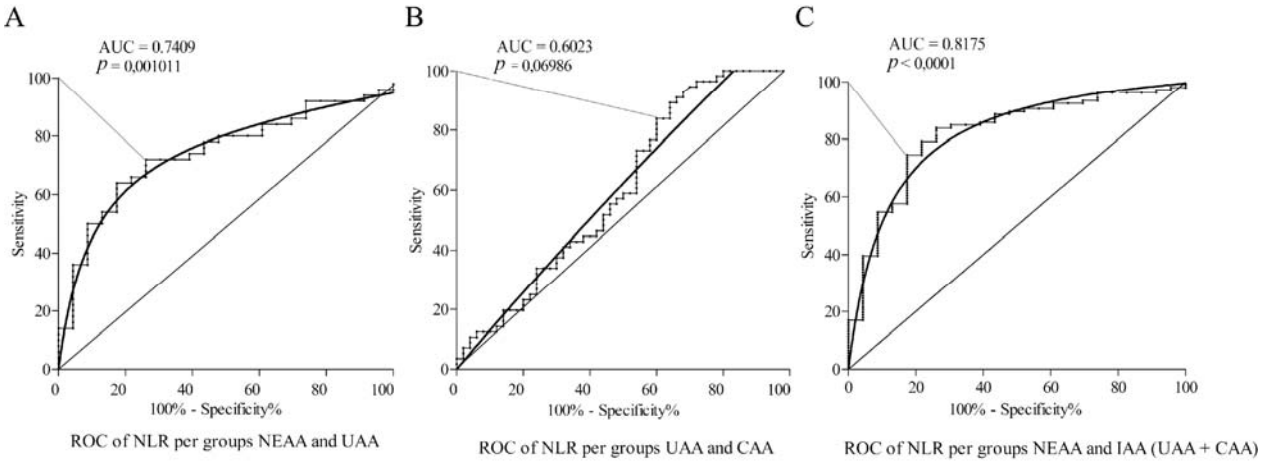

Fig. 8 – Receiver Operating Characteristic (ROC) curves for formed histopathological groups: A) ROC curve for the normal/early acute appendicitis (NEAA) group and the uncomplicated acute appendicitis (UAA) group (sensitivity 71.43%, specificity 73.91%); B) ROC curve for UAA group and the complicated acute appendicitis (CAA) group (sensitivity 83.93%, specificity 40.82%); C) ROC curve for the group NEAA and IAA (UAA+CAA) group (sensitivity 74.53%, specificity 82.61%).

Discussion

Reliable diagnosis of AA and proper indication for surgical intervention remain a challenge in everyday pediatric medical practice. Still, there is no clear boundary between conservative and surgical treatment. As prevention of advanced inflammation and perforation, operative approach of early appendicitis is accepted as a routine surgical practice. In this manner, patients are introduced into the risk for perioperative and postoperative complications.

Reliability in diagnosis of AA is improved by combining clinical, laboratory and imaging methods. However, imaging methods are time consuming and related to radiation while the echosonography is usually insufficiently informative [7]. So, physical examination with a combination of laboratory tests is the basis for diagnosis. Leukocytes, neutrophils, and CRP are commonly used, although none of these laboratory parameters show sufficient sensitivity and specificity [8, 9].

Neutrophils are important types of cells in appendicitis, which is confirmed by histopathological reports. They represent one of the first lines of defense against penetrating agents such as bacteria. These cells are capable of phagocytosis, secretion of lytic enzymes and production of free oxygen radicals with high antimicrobial potential. Activation of neutrophils is a two-stage process triggered by bacteria and their products on cytokines and chemokines. The number of neutrophils in the blood during the inflammatory process growing by mobilization of marginal pool and then from the bone marrow, what is proportional to the extent of inflammation. Lymphocytes are immunocompetent cells that coordinate defensive responses and assist in neutrophil activation. In gangrenous form of AA, significant lymphopenia may occur. Its pathophysiological mechanism is not enough understood [10, 11]. In accordance with this, the increase in the value of NLR in developed form of AA occurs as a result of the increase in the number of neutrophils as well as of reducion of the lymphocytes number.

In our study, a statistically significant difference was recorded among NLR in three samples of blood taken preoperatively and on 1st and 3rd postoperative values, in the total of patients as well as in histopathological gender groups. Except in the NEAA group, all records showed continuous decline of NLR values. Increase of NLR value in the NEAA group on the 1st postoperative day could be result of surgical trauma. The most prominent decline of NLR values was in the CAA group, what may be explained by the highest extent of inflamation in this group and its attenuation after surgery.

Preoperative difference in NLR values among the groups was higher than on the 1st and 3rd postoperative day. This difference was most pronounced between the NEAA group and the other two groups, while smaller between the UAA and CAA group. This is probably due to developing inflammatory process in preoperative phase in the UAA group and the CAA group, while it was in the phase of recovery on the 1st and the 3rd day after surgery, this difference was

smaller due to stabiliyation of immunological condition. These preoperative differences could be of additional diagnostic importance in AA. On the 3rd postoperative day, difference between NLR values in the NEAA group and the UAA group was lost, while in the difference between CAA and the other 2 groups retained statistical significance, probably because of longer period needed for inflammation reduction in the CAA group where the inflammation was intense.

According to the original publication, PAS as a diagnostic tool had a sensitivity of 100% and specificity of 92% for the diagnosis of AA. PAS may have a value from 1 to 10, scoring as the following: migration of pain – 1, anorexia – 1, nausea/emesis – 1, tenderness in right lower quadrant – 2, cough/percussion and hop tenderness – 2, pyrexia – 1, leukocytosis – 1 and polymorphonuclear neutrophilia – 1 [12]. After the subsequently conducted trials, diagnostic value of PAS has been significantly lower [13–15]. NLR in our sample correlated with PAS on the total sample and by histology and gender groups. However, the sensitivity and specificity of NLR for differentiating negative and positive AA in our study were better than PAS. The parameters of PAS related to pain are subjective and unfavorable for assessment due to poor cooperation of younger children during physical examination, what reduces accuracy of PAS.

The clinical presentation of AA is individual and usually depends on the child's age, location and degree of inflammation of the appendix. Different PSD in histopathological groups in our study registered in all three groups, unlike in the previous studies where the PSD was similar in the UAA and the CAA group and clear distinct in the NEAA group [15]. In our study, a strong negative correlation between NLR and PSD was obtained. This could be explained by the fact that advanced forms of the disease quickly lead to the full clinical presentation of AA and decision for surgical treatment. This clinical presentation includes a pronounced neutrophilia sometimes followed by lymphopenia and resulting with increase of NLR.

In our investigation, there was the correlation between NLR and CRP on the 1st and the 3rd postoperative day, but not preoperatively. CRP is protein of acute inflamatory phase and laboratory parameter that along with positive physical findings and radiological findings may have good diagnostic value in AA. However, as an isolated parameter it is not useful because of low specificity [8]. Laboratory parameters which include the value of neutrophils, such as absolute neutrophil count, percentage of neutrophils in the leukocyte formula and NLR, are considered as even better diagnostic parameters in AA because neutrophils rise faster than CRP which needs time for synthesis in the liver [16]. Some studies showed that NLR appears to have greater diagnostic accuracy then the WBC count [17]. The first increase in CRP in blood records 12 hours after the occurrence of inflammation while peak plasma concentration reaches between 24 to 48 hours [18]. Another studies suggest that CRP is an important diagnostic agent for perforated AA but not for AA in general [19]. Our findings confirm a faster NLR increase and delayed increase of CRP in pediatric AA. In this way, increase of NLR may suggest earlier phases of AA which can be successfully treated with antibiotics.

Cutoff value of NLR distinguishing the NEAA from the UAA may be a good clinical indicator for the introduction of antibiotic therapy [20]. In our group of patients, this value was 5.06 what is significantly higher than the values obtained in other studies conducted in children [9, 15] and in adults or mixed groups of patients [21, 22]. At the same time, our cutoff value has much better sensitivity and specificity. On the other hand, according to our results, NLR could not be so reliable diagnostic tool for distinguishing the UAA from the CAA, what should be of great value for making a decision on an urgent surgery of AA. The value we received for distinguishing these two groups was 6.325, but with a lower sensitivity and specificity. This is not in accordance with the results of some studies in which the cutoff value to distinguish the UAA and the CAA had significantly higher sensitivity and specificity [21]. Combining the UAA and the CAA group we got a new group of acute inflamed appendix. Cutoff value of NLR distinguishing the NEAA group from this new group, which means between negative and positive appendectomy was 6.14 with high sensitivity and specificity. This value is very similar to that distinguishing the UAA from the CAA, taking into account limitation that we had small number of patients in the NEAA group comparing to newly defined group of acute inflamed appendix.

Conclusion

NLR well monitors and reflects inflammatory process progression in AA in children and could be useful in the clinical stage differentiation of the disease. We found strong positive correlation of NLR with CRP as well as with PAS, while NLR negatively correlates with PSD. Values of NLR are changing earlier then the CRP values, which means its better reactivity and, therefore, usefulness of NLR in early diagnosis of AA is better than that of CRP. Values of NLR for distinguishing the disease phases are much higher in our study then in the earlier condacted invastigations, with cutoff of 5.06 for the UAA and 6.325 for the CAA.

Considering good specificity and sensitivity, easy availability and use in practice, NLR should be used with other diagnostic methods in the diagnosis of acute appendicitis in children.

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Evaluation of apically extruded debris during removal of gutta-percha and Resilon™ using different instrumentation techniques

Procena apikalno ekstrudiranog debrisa tokom uklanjanja gutaperke i Resilona™ primenom različitih tehnika instrumentacije

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Abstract

Background/Aim. Apical extrusion of material is considered as one of the very important factor for endodontic treatment success. Microorganisms, necrotic tissue, filling material and irrigants, which can be extruded apically during endodontic retreatment, may jeopardize the success of the therapy. The aim of this *in vitro* study was to quantitatively evaluate the amount of apically extruded debris during endodontic retreatment of teeth obturated with two different materials, using one hand and three rotary instrumentation techniques. **Methods.** Ninety-six extracted single-rooted teeth were endodontically treated and enlarged to size 40 using BioRaCe system, and then randomly divided into 8 groups of 12 specimens each. Half of the specimens were obturated with gutta-percha and AH Plus® sealer and another half with RealSeal SE system, using lateral condensation technique. Retreatment was performed using: Hedström files; ProFile rotary files; ProTaper Retreatment system and D-RaCe system. Apically extruded debris was collected in pre-weighed Eppendorf tubes and evaluated using an electronic microbalance. Data were analyzed statistically using *t*-test and analysis of variance. **Results.** In the AH Plus/gutta-percha group, all tested rotary instruments gave significantly less extruded debris compared with Hedström files ($p < 0.05$). In theRealSeal group, there was significant difference between D-RaCe and Hedström instruments ($p < 0.05$). **Conclusion.** All retreatment techniques resulted in apical extrusion. D-RaCe system produced significantly less extruded debris compared to hand files. No significant difference was found when comparing two instruments specially designed for retreatment: D-RaCe and ProTaper systems.

Key words:
root canal filling materials; gutta-percha; pit and fissure sealant; dental instruments; methods.

Apstrakt

Uvod/Cilj. Apikalna ekstruzija materijala se smatra jednim od veoma važnih faktora za uspešnost endodontske terapije. Mikroorganizmi, nekrotično tkivo, opturacioni material i irigansi koji mogu biti ekstrudirani apikalno tokom endodontskog retretmana mogu ugroziti uspeh terapije. Cilj ovog istraživanja je bio da se kvantitativno proceni količina apikalno ekstrudiranog debrisa tokom endodontskog retretmana zuba opturiranih sa dva različita materijala, primenom jedne ručne i tri rotirajuće instrumentacione tehnike u *in vitro* uslovima. **Metode.** Ukupno 96 ekstrahovanih jednokorenih zuba su endodontski lečeni i proširenj primenom BioRaCe sistema (završni proširivač 40/0.04), a zatim nasumično podeljeni u osam grupa od po 12 zuba. Polovina uzoraka je bila opturirana gutaperkom i AH Plus® silerom, a druga polovina RealSeal SE sistemom, korišćenjem tehnike hladne lateralne kondenzacije. Retretman je obavljen primenom: Hedström ručnih instrumenata, ProFile rotirajućih instrumenata, ProTaper Retreatment sistema i D-RaCe sistema. Apikalno ekstrudirani debris je sakupljan u prethodno izmerene Eppendorf epruvete a procena je obavljena primenom elektronske mikrovage. Podaci su statistički analizirani primenom *t*-testa i analizom varijanse. **Rezultati.** U AH Plus/gutaperka grupi, kod svih ispitivanih rotirajućih instrumenata dobijeno je značajno manje ekstrudiranog debrisa u poređenju sa Hedström instrumentima ($p < 0,05$). U RealSeal grupi, bila je prisutna statistički značajna razlika između D-RaCe i Hedström instrumenata ($p < 0.05$). **Zaključak.** Apikalna ekstruzija debrisa je bila prisutna kod svih ispitivanih tehnika retretmana. Primenom D-RaCe sistema dobijeno je značajno manje ekstrudiranog debrisa u poređenju sa ručnim instrumentima. Pri upoređivanju instrumenata specijalno dizajniranih za retretman – D-RaCe i ProTaper sistema, nije utvrđeno prisustvo statistički značajne razlike.

Ključne reči:
zub, materijali za punjenje korenskog kanala; gutaperka; zub, zalivači jamica i fisura; stomatološki instrumenti; metodi.

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Introduction

Irritants such as microorganisms, necrotic tissue, filling material and root canal irrigants can be extruded apically during endodontic retreatment. Apical extrusion of material is considered as one of the very important factors for endodontic treatment success. A number of studies over the past decades have shown transportation of apically extruded material to some degree [1–4]. The amount of extruded debris may vary depending on the techniques of preparation and design of the instruments used [5–8]. Therefore, appropriate retreatment technique should be selected to remove the preexisting filling material as much as possible while minimizing the amount of apical extrusion [3].

Numerous studies showed various outcomes concerning the amount of apically extruded debris when hand instruments were used compared to rotary. Bharathi et al. [9] measured significantly less extruded debris after retreatment with ProFile instruments, compared to manual Hedström instruments. In another study, rotary instruments (Mtwo and Reciproc instruments) proved to be more successful than Hedström instruments while removing gutta-percha/AH Plus sealer [10].

A new root canal filling material, RealSeal SE system (SybronEndo, Orange, CA, USA) was recently introduced to the market. It consists of Resilon™ cones and Realseal SE self-etch sealer. Resilon™ like gutta-percha is a biocompatible filling material and the clinical outcome and obturation quality are similar, too[11]. Only few authors have investigated the importance of extrusion in the periapical tissues during retreatment of Resilon™ based obturation materials [12, 13].

Also, no studies can be found in the current literature concerning the amount of apically extruded debris during retreatment procedure on samples obturated with RealSeal SE system using D-RaCe rotary files.

The aim of this *in vitro* study was to compare the amount of apically extruded material during endodontic retreatment of teeth obturated with two different materials, using one hand and three rotary instrumentation techniques [1].

Methods

Specimen selection

This *in vitro* study was carried out on 96 human mandibular premolars, freshly extracted for orthodontic or periodontal reasons. Teeth with mature apices and straight root canal (< 10º) were selected according to the Schneider method [14]. Samples with incomplete root formation, the presence of external resorption, two or more root canals, localized or diffuse calcifications were excluded from the study. Periodontal tissue, organic debris and calculus were mechanically removed from the root surface after immersion of the samples in a 2.5% sodium hypochlorite solution for 8 hours.

Root canal preparation and obturation

After accessing the cavity and removing the pulp tissue, the canal patency and working length determination was established with a size 10 K-type file (Dentsply Maillefer, Ballaigues, Switzerland). Crowns were cut to a level of 15 mm from the apical foramen in order to standardize the amount of filling material. The working length of each canal was visually determined to 1 mm short of the major apical foramen. Root canal preparation was performed with a crown-down technique using rotary Bio-RaCe instruments (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland). For preparation of the coronal third instrument BR0 (25/0.08) was used. Middle third was instrumented with BR1 (15/0.05), BR2 (25/0.04) and BR3 (25/0.06) instruments, and apical third with BR4 (35/0.04) and BR5 (40/0.04) instruments. Each instrument was used according to the protocol allowed (4 times for simple root canal anatomy as recommended). Instruments were driven by low-torque rotary engine motor Rooter (FKG Dentaire SA, La Chaux de Fonds, Switzerland), with the torque control set to 1N/cm and at constant speed of 600 rpm, and inserted in root canal in movement. Canal^{+} (Septodont, Saint-Maur-des-Fossés, France) was used as lubricant and chelating agent. Apical third patency was enabled using a size 10 K-type file between every rotary instrument. Each root canal was irrigated with 2 mL of 3% NaOCl solution between all instrument changes. Final irrigation was carried out with 17% ethylenediamine tetraacetic acid (EDTA) for smear layer removal. The residual irrigants were removed with 9 mL of distillated water. After instrumentation and irrigation, roots were dried with sterile paper points.

The roots were randomly divided into 8 groups of 12 specimens each (Table 1). Forty-eight teeth (groups 1, 2, 3, and 4) were obturated with gutta-percha and AH Plus® sealer (Dentsply DeTrey, Konstanz, Germany) using lateral condensation technique. Another forty-eight specimens (groups 5, 6, 7 and 8) were filled with RealSeal SE system (SybronEndo, Orange, CA, USA), using lateral condensation technique. The coronal surface of RealSeal groups was light cured for 40 s. Cavities were sealed with GC Fuji II (GC

Table 1

Experimental groups

Specimens (n)	Retreatment technique	Filling material
12	Hedström	RealSeal SE
12	ProFile	Gutta-percha/AH Plus®
12	PTUS	RealSeal SE
12	D-RaCe	Gutta-percha/AH Plus®
12	Hedström	Gutta-percha/AH Plus®
12	ProFile	RealSeal SE
12	PTUS	Gutta-percha/AH Plus®
12	D-RaCe	RealSeal SE

PTUS – ProTaper Universal Retreatment System.

[1]**Note:** Resilon is a trademark of Resilon Research, LLC.

Corporation, Tokyo, Japan). Teeth were radiographed in bucco-lingual and mesio-distal directions to evaluate the quality of obturation. Roots were then incubated in saline at 37°C (INCUCELL, MMM Group, München, Germany) for 3 weeks in order to complete setting of filling material.

Retreatment techniques

In groups 1 and 5 the obturation material was removed using Gates-Glidden drills and Hedström files (VDW GmbH, München, Germany). The coronal third of the root canal fillings were removed using Gates-Glidden drills sizes from 6 to 4 at 300 rpm using crown-down technique. Hedström files sizes 35, 30 and 25 were used sequentially in a crown-down manner for removal of the filling material from the middle and apical thirds until working lenght was achieved. Apical enlargement was performed with Hedström files up to size #40.

In groups 2 and 6 the obturation material was removed using ProFile rotary instruments **(**Dentsply Maillefer, Ballaigues, Switzerland), with crown-down technique following the manufacturer's instruction. Instruments were inserted into the canal in constant rotation with light apical pressure, with rotation speed set at 300 rpm. For removal of filling material from coronal third, three instruments were used consecutively: ProFile size 3 and 2 orifice shapers O.S.3 (40/0.06), O.S.2 (30/0.06) and 25/0.06; for the middle third of the root canal: 20/0.06 and 25/0.04 and for the apical third: 20/0.04, 25/0.04 with apical enlargement to size 40, 0.04 taper.

In groups 3 and 7 the obturation material was removed using ProTaper Universal Retreatment System (PTUS) **(**Dentsply Maillefer, Ballaigues, Switzerland) and with the crown-down technique, as recommended by the manufacturer. Each of three instruments D1 (30/0.09), D2 (25/0.08) and D3 (20/0.07) were sequentially used each for every third of the root canal and were manipulated in a brushing action with lateral pressing movements. Rotational speed was set at 500 rpm, and torque control at 1 N/cm as recommended. After complete removal of filling material, the final canal preparation was performed with finishing ProTaper instruments F3 (30/0.09) and F4 (40/0.06) at working length.

In groups 4 and 8 the obturation material was removed using D-RaCe rotary system (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) and with the crown-down technique, as recommended by the manufacturer. DR1 (30/0.10) instrument was used for removal of filling material from the coronal third of the root canal. Instrument was inserted into the canal in constant movement, with rotational speed set at 1000 rpm, and torque control set at 1.5 N/cm as recommended, using rotary engine motor Rooter (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland). For removal of filling material from the middle and apical thirds, DR2 (25/0.04) instrument was used, with light apical pressure, with rotational speed set at 600 rpm, and torque control set at 1 N/cm as recommended. Further apical preparation was performed with BioRaCe instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland) BR3 (25/0.06), BR4 (35/0.04) and BR5 (size 40/0.04) at 600 rpm.

During retreatment procedure all samples were irrigated with 3% NaOCl solution between instruments. Canal^{+} (Septodont, Saint-Maur-des-Fossés, France) was used as the lubricant during instrumentation. Final irrigation was performed with 17% EDTA solution folowed by distillated water. Canal patency was preserved by stainless steel K files, which was used to establish a glide path before introducing next instrument. Each instrument was cleaned from adherent debris after its use. All instruments were discarded after use in three root canals exept DR2 instrument, which is recommended for single use. Rotary NiTi-instruments were driven by low-torque rotary engine motor Rooter (FKG Dentaire SA, La Chaux de Fonds, Switzerland), according to the manufacturer's instructions. Retreatment was considered to be finished when no more gutta-percha or Resilon™ could be seen on the instruments, and the walls of the canal were smooth and free of visible debris.

Debris collection and measurement

Apically extruded debris was collected using a modification of the experimental model described by Myers and Montgomery [15] (Figure 1). Every tooth was secured for instrumentation and debris collection by passing the sample through an opening in a rubber stopper. Eppendorf tube, in which all of the apically extruded debris was collected, was placed below root, so the root apex hung within the receptor tube. Removable rubber stopper, together with specimen and adjusted Eppendorf tube, was placed on the opening of 20 mL volume glass vial. The needle was inserted in the rubber stopper of glass vial to equalize the internal and external pressures. All vials were covered with cofferdam in order to prevent the operator from viewing debris extrusion during retreatment, to ensure data objectification. No contact with collecting tube was possible. Before retreatment, each Eppendorf tube was marked and weighed using the microbalance (OHAUS Pioneer^{TM} Balance, PA214C, Parsippany, NJ, USA).

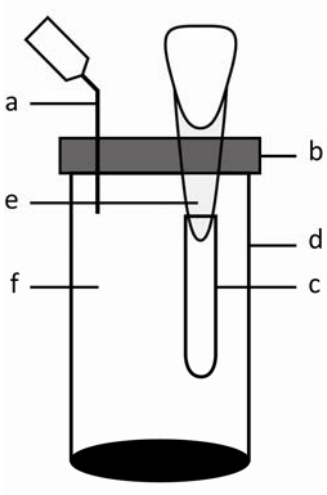

Fig. 1 – Assembly prepared for the evaluation of apically extruded debris during retreatment. a) disposable needle inserted in the rubber stopper of glass vial to equalize the internal and external pressures; b) rubber stopper to hold the root; c) Eppendorf tube for debris collection; d) glass vial acting as a holder to this assembly; e) root; f) air.

After the retreatment procedure was finished, teeth were separated from the receptor tube and each apical root surface was washed with 0.2 mL of distilled water into the tube, for removing debris still attached to the root. After the removal of the specimens, the collecting tubes were stored in an incubator (INCUCELL, MMM Group, München, Germany) at 70°C for 5 days to evaporate the moisture before weighing the dry debris. An electronic microbalance (OHAUS Pioneer™ Balance, PA214C, Parsippany, NJ, USA) with an accuracy of 0.1 mg was used for weighing the tubes. Three consecutive measurements were taken for each tube, and the mean value was recorded. The dry weight of extruded debris was calculated by subtracting the weight of the empty tube from the weight of the tube containing the extruded debris.

Statistical analysis

Statistical analysis of the amount of the extruded debris was performed with *t*-test with Bonferroni correction and analysis of variance (ANOVA) combined with *post hoc* Scheffe's multiple comparison tests. Statistical significance level was established at 0.05.

Results

When comparing the amount of apically extruded debris concerning the obturation material for each instrument individually, *t*-test showed no significant difference (Table 2).

In samples obturated with gutta-percha and AH Plus® sealer, ANOVA test showed significant difference in the amount of extruded debris between the tested instruments (Table 3). Results of the post hoc Scheffe's test showed no significant difference between tested rotary instruments. D-RaCe, ProFile and ProTaper instruments gave significantly less extruded debris compared to Hedström hand files (Table 3).

In samples obturated with RealSeal SE system, ANOVA test showed significant difference for the amount of extruded debris between the tested instruments (Table 3). Post hoc Scheffe's test showed significant difference only between D-RaCe and Hedström instruments. A significantly lower amount of debris was measured when D-RaCe instruments were used compared to Hedström instruments (Table 3).

Discussion

Apically extruded debris is considered as one of the reasons for endodontic treatment failure, but also has a large impact on retreatment procedure failure. It has been convincingly demonstrated that periapical pathological lesions consistent with apical periodontitis, may be caused by foreign bodies present within the periapical tissues [16]. Also, the inflammatory response is likely to be more severe with the increase of the amount of apically extruded debris [17].

Methods of evaluation of apically extruded debris are different in various studies, but some can be comparable. Few studies used visual evaluation, directly or on radiography [12, 18–23]. This is a qualitative method of evaluation where one can't have insight into the amount of material extruded through the apical foramen. Compared with an analytical balance, visual examination is not an accurate method of evaluation [24].

In the present study, quantitative evaluation method of apically extruded debris provided numerical results comparable to results of other authors [3, 9, 10, 25, 26]. A significantly higher amount of apically extruded debris was recorded during retreatment when hand instruments were used in comparison to rotary instruments, which is consistent with results of other studies [3, 9, 10]. Bharathi et al. [9] measured significantly less extruded debris after retreatment with ProFile instruments, compared to hand Hedström files and Hedström files in com-

Table 2

Comparison of apically extruded debris regarding the filling material for each instrument individually

Filling material/Retreatment techniques	DF	*t*-value	*p*-values
AH Plus® gutta-percha (Hedström)/RealSeal (Hedström)	22	1.76	0.0927
AH Plus® gutta-percha (ProFile)/RealSeal (ProFile)	22	0.71	0.4854
AH Plus® gutta-percha (PTUS)/RealSeal (PTUS)	22	0.35	0.7328
AH Plus® gutta-percha (D-RaCe)/RealSeal (D-RaCe)	22	1.72	0.1002

PTUS – ProTaper Retreatment System; DF – degree of freedom.

Table 3

Weight of apically extruded debris during retreatment of samples obturated with gutta-percha and AH Plus® sealer and with RealSeal SE systems

Retreatment technique	Weight of debris (g), $\bar{x} \pm SD$	
	Gutta-percha/AH Plus® sealer	RealSeal System
Hedström	0.0069 ± 0.0016^{a}	0.0056 ± 0.0019^{a}
ProFile	0.0044 ± 0.0028^{b}	$0.0038 \pm 0.0016^{b, a}$
PTUS	0.0044 ± 0.0028^{b}	$0.0037 \pm 0.0016^{b, a}$
D-RaCe	0.0036 ± 0.0018^{b}	0.0025 ± 0.0014^{b}
ANOVA	$p = 0.0007$	$p = 0.0005$

$\bar{x}$ – arithmetic mean; SD – standard deviation; PTUS – ProTaper Retreatment System. Scheef's post hoc test – means followed by the same letters are not significantly different ($p > 0.05$).

bination with a solvent. Also, rotary instruments (Mtwo and Reciproc) proved to be more successful than Hedström files concerning the amount of apically extruded debris [10].

In the study of Topçuoğlu et al. [27], all evaluated retreatment techniques caused the apical extrusion of debris. Investigators concluded that hand files produced significantly more debris then ProTaper, D-RaCe, and R-Endo rotary systems, while there was no statistical difference among rotary systems, which is in compliance with the results of the present study.

Findings of previous studies indicate that the rotary instruments tend to direct debris coronary rather than apically [3, 9, 10, 25]. It can be assumed that the crown-down technique, which enables faster elimination of gutta-percha from coronal third of the root canal, reduced possibility of debris extrusion during the removal of the remaining sealer from the apical third, allowing evacuation of the contents in the coronal direction. The reason why crown-down technique was used in retreatment procedure is the fact that it has been proved for less debris extrusion when compared to other techniques [1].

However, it should be noted that there are conflicting results regarding the amount of extruded material comparing hand vs. rotary instruments. A study conducted by Somma et al. resulted with significantly greater amount of apically extruded debris when rotary instruments were used. This disagreement may be referred to the scoring method that was used to assess the amount of debris, which may be less sensitive than quantitative evaluation of debris that was used in the present study.

The design of instruments might have an impact on the quantity of extrusion through the apical foramen [1, 3, 5, 7, 8, 28]. Two out of three rotary instruments used in the present study, are specially designed for endodontic retreatment. When using those instruments in the retreatment procedure, less transportation of root canal content in periapical tissue should be expected. D-RaCe system consists of DR1 and DR2 instruments, which are designed with alternating cutting edges as well as a triangular cross section. The first instrument, D1, as DR1 instrument in PTUS, has an active working tip to facilitate the initial penetration of the filling material.

According to Duncan and Chong [29], the amount of the extruded material does not necessarily depend on the technique used for root canal filling removal. Some of the authors have concluded that the amount of apically extruded material was not significantly different after comparing various instrumentation techniques [18–23, 26, 27]. However, all of these studies used visual evaluation of the extruded debris.

Only few studies have compared the amount of apically extruded material during retreatment of teeth filled with Resilon™ system [12, 13]. In the present study, the measured amount of apically extruded material was significantly lower after removing RealSeal system from the root canal, compared to the amount of debris measured after the removal of gutta-percha and AH Plus® sealer, but without any significant difference. This indicates that the type of filling material did not play a significant role in the amount of apically extruded debris, which is in compliance with the results of other authors [12].

In Al-Haddad and Che study [13], during removal of RealSeal™, no significant difference was found between groups regarding obturation technique or type of files used during retreatment.

Conclusion

In the present study, apical extrusion was recorded during retreatment of teeth obturated gutta-percha/AH Plus® sealer and RealSeal SE system and the lowest amount of debris was measured after the use of D-RaCe instruments, and the highest amount when using Hedström files, with a significant difference ($p < 0.05$). There was significantly less debris when using rotary instruments versus hand instrumentation technique ($p < 0.05$). Between tested rotary instruments, no difference in apically extruded debris was found ($p > 0.05$). Further research on the amount of apically extruded debris is required, with the application of instruments specially designed for removal of different obturation materials, on both straight and curved root canals.

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Differences in quality of life between patients with severe hip and knee osteoarthritis

Razlike u kvalitetu života između pacijenata sa teškom artrozom kuka i kolena

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Abstract

Background/Aim. Osteoarthritis (OA) is the clinical manifestation of degenerative joint changes. The aim of this study was to investigate differences in quality of life (QoL) between patients with severe hip and knee OA. **Methods**. This is the cross-sectional study of 195 patients (average age 63.2 ± 11.1 yrs), with a diagnosis of OA of the hip and knee that were assigned to receive a total hip or knee replacement. The patients were divided into three groups in relation to localization of OA. The first group included patients with hip OA; the second group consisted of patients with knee OA and the third group with both hip and knee OA. Demographic and clinical data were collected for each patient. We measured health-related quality of life (QoL) by Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires. Statistical significance of differences was at the level of $p < 0.05$. **Results**. The best QoL was in the group of knee OA (42.7 ± 11.3) and the worst in the group with both hip and knee OA patients (35.8 ± 12.7). QoL assessed by WOMAC score and the domain of physical function were significantly different among three groups of patients with OA ($F = 5.377$, $p < 0.01$ and $F = 5.273$, $p < 0.01$) respectively). Results of three multiple linear regression models where WOMAC score was dependent variable and age, body mass index (BMI), social class, pain, stiffness, physical function, hypertension, cardiomyopathy, diabetes mellitus were independent variables, have shown that QoL was statistically significantly associated with pain and physical function in the hip and knee OA groups, whereas in the group with both hip and knee OA patients, QoL was associated with BMI, pain, physical function and diabetes mellitus. **Conclusion**. QoL of patients with severe hip and knee osteoarthritis in relation to localization was significantly different. QoL in severe hip and knee OA patients was significantly associated with pain and physical function, but in patients with both hip and knee OA QoL was also associated with BMI and diabetes mellitus.

Key words:
osteoarthritis; hip; knee; quality of life.

Apstrakt

Uvod/Cilj. Osteoartritis je klinička manifestacija degenerativnih promena u zglobu. Cilj ove studije je bio da se istraži postojanje razlika u kvalitetu života (QoL) između bolesnika sa teškom artrozom (OA) kuka i kolena. **Metode**. Studijom preseka obuhvaćeno je 195 bolesnika (prosečne starosti 63.2 ± 11.1 godina) sa dijagnozom artroze kuka ili kolena, kojima je bila indikovana totalna artroplastika kuka ili kolena. Bolesnici su podeljeni u tri grupe u odnosu na lokalizaciju artroze. Prvu grupu su činili bolesnici sa artrozom kuka, drugu sa artrozom kolena i treću sa artrozom i kuka i kolena zajedno. Za svakog bolesnika smo beležili demografske i kliničke podatke. Kvalitet života ovih bolesnika merili smo pomoću Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) upitnika. Za statističku značajnost razlika uzimali smo nivo od $p < 0.05$. **Rezultati**. Najbolji QoL je bio u grupi bolesnika sa OA kolena (42.7 ± 11.3), a najlošiji u grupi bolesnika sa OA kuka i kolena zajedno (35.8 ± 12.7). QoL procenjen WOMAC skorom je bio značajno različit između tri grupe bolesnika sa artrozom ($F = 5.377$, $p < 0.01$), kao i domen fizičke funkcije ($F = 5.273$, $p < 0.01$). Rezultati tri modela multiple linerane regresije gde je WOMAC skor zavisna varijabla, a godine starosti, indeks telesne mase (BMI), socijalni status, bol, ukočenost, fizička funkcija, arterijska hipertenzija, miokardiopatija, dijabetes melitus nezavisne varijable, pokazali su da je QoL procenjen WOMAC skorom statistički značajno udružen sa bolom i fizičkom funkcijom u prvoj (OA kuka) i drugoj (OA koljena) grupi bolesnika, dok je u trećoj grupi bolesnika (sa OA kuka i koljena zajedno), WOMAC skor bio udružen sa BMI, bolom, fizičkom funkcijom i dijabetes melitusom. **Zaključak**. Kod bolesnika sa teškom OA kuka i kolena, QoL je, u odnosu na lokalizaciju, bio značajno različit. Bol i fizička funkcija su značajno udruženi sa QoL bolesnika sa teškom OA kuka i kolena. U grupi bolesnika sa OA kuka i kolena zajedno, QoL je bio značajno udružen i sa BMI i dijabetesom melitusom.

Ključne reči:
osteoartritis; kuk; koleno; kvalitet života.

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Introduction

Osteoarthritis (OA) is the clinical manifestation of degenerative joint changes [1–3]. The final stage is the phase of definite disability with severe pain, very restricted movements, greater functional disturbances and worse quality of life (QoL). OA is the most common joint disease, mainly affecting middle-aged and elderly people [4]. People with OA of the knee or hip experience pain and deconditioning that may lead to disability. Treatment goals include pain control, maximizing functional independence, and improving QoL within the constraints imposed by both OA and comorbidities [5]. Outcome measures can help us evaluate the functional status of the patients and they provide an objective tool for assessing the severity of impairment, functional disability and handicap at personal and community levels [6–9]. The Western Ontario and McMaster Osteoarthritis Index (WOMAC) questionnaires measure health-related QoL and specific outcome measures for OA. It has been developed for assessing the severity of knee and hip OA and it has shown greater responsiveness to change over time [8].

There are reports of differences between hip and knee severe OA [10, 11]. Differences between hip OA and knee OA patients in health state and QoL may have substantial direct costs to the health care system and indirect costs to society [10]. Results of this study could have clinical importance in rehabilitation and improve understanding of impact of various factors on differences in hip OA and knee OA patients. Studies of OA patients and their QoL published in recent years have reported that there are differences between hip OA and knee OA [10], but fewer of them were related to differences in QoL of hip and knee OA patients in relation to localization (hip, knee, both hip and knee) comorbidity, sex, age body mass index (BMI), and social class [12, 13].

Therefore, the aim of this cross-sectional study was to investigate differences in QoL in patients with severe hip OA and knee OA after adjusting for potential confounders.

Methods

Patients

In this cross-sectional study of 195 consecutive patients (average age of 63.2 ± 11.1 yrs) with diagnosis of OA of the hip or knee, according to the American College of Rheumatology [11], who had both clinical and radiographic evidence of severe OA (and were assigned to receive a total hip or knee replacement), participated and completed this study in the Department of Orthopaedics. Patients were divided into three groups (Table 1): the first group with hip OA (65 patients, average age of 63.2 ± 10.7 yrs), the second group with knee OA (65 patients, average age of 64.1 ± 11.3 yrs) and the third group with both hip OA and knee OA (65 patients, average age of 62.2 ± 11.5 yrs). A standardized joint examination and radiographs in two projections of the pelvis and knee with the participant standing, were obtained by a standardized procedure. Demographic and clinical data were collected for each patient including age, gender, height, weight, BMI, location of OA, social class and comorbidity (hypertension, cardiomyopathy, diabetes mellitus). Individuals with contra lateral limb injury or abnormality, major neurological or cardiovascular disorder and medical conditions limiting activity were excluded from the study. BMI (kg/m2) was calculated as follows: subjects were classified as normal-weight (BMI of 18.5–24.9 kg/m2), overweight (BMI of 25.0–29.9 kg/m^2), or obese (BMI of >/= 30 kg/m^2). Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, by using standardized equipment and procedures [14]. Socio-economic level was scored by "1" (low - patients have no health insurance and have low income), "2" (middle - patients have health insurance and average income) and "3" (high - patients have health insurance and above average income).

Informed consent was obtained from all subjects. All parameters that were collected (sex, age, comorbidity, social

Table 1

Characteristics of the study participants (n = 195)

Parameters	Group I (Hip osteoarthritis)	Group II (Knee osteoarthritis)	Group III (Hip and knee osteoarthritis)	Total	Differences among three groups (*p*)
Age (years), $\bar{x}$ ± SD	63.2 ± 10.7	64.1 ± 11.3	62.2 ± 11.5	63.2 ± 11.1	0.628
Osteoarthritis					
right side	35 (13.5)	34 (13.1)	76 (29.2)	145 (55.8)	0.672
left side	30 (11.5)	31 (11.9)	54 (20.8)	115 (44.2)	
Sex					
female, n (%)	41 (21.0)	41 (21.0)	42 (21.6)	124 (63.6)	0,978
male, n (%)	24 (12.3)	24 (12.3)	23 (11.8)	71 (36.4)	
Social class					
low, n (%)	4 (2.1)	2 (1)	2 (1)	8 (4.1)	0.480
middle, n (%)	57 (29.2)	58 (29.8)	54 (27.7)	169 (86.7)	
high, n (%)	4 (2)	5 (2.6)	9 (4.6)	18 (9.2)	
BMI (kg/m^2), $\bar{x}$ ± SD	31.2 ± 4.6	31.5 ± 4.8	31.2 ± 5.0	31.3 ± 4.8	0.935
Comorbidity					
hypertension, n (%)	3 (1.5)	7 (3.6)	9 (4.6)	19 (9.7)	0.688
cardiomyopathia, n (%)	3 (1.5)	3 (1.5)	5 (2.6)	11 (5.6)	
diabetes mellitus, n (%)	1 (0.5)	1 (0.5)	3 (1.6)	5 (2.6)	

Note: Values are expressed as mean ($\bar{x}$) ± standard deviation (SD) or numbers (%).
BMI – body mass index.

class and WOMAC Index) are part of the regular hospital procedures for all patients that were assigned to receive a total hip or knee replacement. Study methods were approved by the institutional Ethics Committee of Institute of Rehabilitation "Dr Miroslav Zotovic", Banja Luka, Bosnia and Herzegovina.

Questionnaire

QoL of patients with OA was measured by disease-specific instrument (WOMAC index). The WOMAC Index is an OA-specific health status instrument; its validity, reliability and responsiveness have been extensively demonstrated [15]. This is a self-administered instrument validated for OA in the legs. It was used to assess QoL of the patients with hip and knee OA as the disease-specific measure. QoL was measured by WOMAC, developed for OA in the hip and knee for each patient in our research.

The WOMAC is a multidimensional index containing 24 multiple choice questions: 5 dimensions for pain, 2 for stiffness, and 17 for physical function (for example regarding the ability to perform activities of daily living). The parameters of the pain were: walking, stair climbing, nocturnal rest and weight bearing. Stiffness included two parameters: morning stiffness and stiffness occurring later in the day. Physical function obtained parameters about descending stairs, ascending stairs, rising from sitting, standing, bending to floor, walking on flat, getting in or out of car, going shopping, putting on socks, rising from bed, taking off socks, lying in bed, sitting, getting on or off toilet, heavy domestic duties, light domestic duties. Each item is represented by a Likert scale between 0 (best health state) and 4 (worst state). Responses were: "none" scoring by "0", slight by "1", moderate by "2", severe by "3" and extreme by "4". Score was = SUM of all points for relevant items. Maximum pain subscore was 20, maximum stiffness subscore 8 and maximum physical function subscore was 68. Minimum total score was 0. Maximum total score was 96. The WOMAC summary score ranged from 0 (no pain or disability) to 96 (the most severe pain and disability). Global (i.e., total) score [16] is determined as sum x 100/96. Each subscale score was transformed to a range from 0 to 100 points, with a score of 100 indicating no pain, dysfunction, or stiffness. QoL was measured using WOMAC questionnaires for each patient with hip and knee OA. WOMAC Index (Serbian version) is part of the regular hospital procedures for all patients that were assigned to receive a total hip or knee replacement. Its linguistics and cultural validity were confirmed by hospital institution before this investigation.

Statistical analysis

Student's t test and Chi-Square test were used to compare baseline performances of the three groups of patients with hip and knee OA. Analysis of variance with single factor (ANOVA) was used to assess differences in QoL between three groups of the patients with OA and Multiple linear regression (in three multiple linear regression models of WOMAC scores) was used to assess the association between QoL measured by WOMAC of three locations (hip, knee, both hip and knee osteoarthritis) where WOMAC score as continued variable was the dependent variable. The independent variables were age, sex, BMI, social class, pain, stiffness, physical function, comorbidities, (hypertension, cardiomyopathy and Diabetes mellitus). Statistical significance of differences was at the level of $p < 0.05$.

Results

Table 1 presents characteristics of the study participants. Average age of the participants in our study was 63.2 ± 11.1 yrs (63.2 ± 10.7 for patients with severe hip OA, 64.1 ± 11.3 for knee OA patients and 62.2 ± 11.5 for the third group of the patients with both hip and knee OA, candidates for arthroplasty). Average age of the patients among these three groups was not statistically significant ($p > 0.05$). In the total sample of patients there were 124 females and 71 males. Difference was statistically significant ($p < 0.001$). In the group with hip and knee OA 41 (21%) of cases were females and 24 (12.3%) of cases were males. In the group with both hip OA and knee OA there were 42 (21.6%) females and 23 (11.8%) males. There was no statistically significant difference among three groups of the patients in relation to sex ($p > 0.05$), but within the each group there was statistically significantly higher number of female than male patients ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively). Thirty-five, or 13.5 % of the patients with hip OA had severe OA on the right side, 30, or 11.5% on the left side (Table 1). Severe knee OA was on the left side in the 31, or 11.9% of the patients and on the right side in 34, or 13.1% of the patients. There were hip OA on the right side in 34, or 13.1% and on the left side 31, or 11.9% , and knee OA on the right side in 42, or 16.1% and on the left side 23, or 8.9% of the OA localisation of the patients with both hip and knee OA. There was no statistically significant difference among the three groups of the patients in relation to a side of the OA. Average value of the BMI was 31.3 ± 4.8 kg/m^2. It was in the range of obesitas in all three groups of patients: the hip OA (31.2 ± 4.6 kg/m^2) the knee OA (31.5 ± 4.8 kg/m^2) and both hip and knee OA (31.2 ± 5 kg/m^2). The differences among the groups were not significant ($p > 0.05$). The majority of patients were in the middle social class: in the hip OA group 57, or 29.2%, in the knee OA patients 58, or 29.8% and in the group with both hip and knee OA 54, or 27.7%. Differences among the three groups of the patients were not statistically significant ($p > 0.05$). Nine, or 4.6% of the patients in the third group had hypertension, 7, or 3.6% in the second and 3 or 1.5% in the first group of the patients. Cardiomiopathy was registered in 3, or 1.5% in the first and second group and 5, or 2.6% in the third group of the patients. Diabetes mellitus was registered in 1, or 0.5% in the hip and knee group and in 3, or 1.6% in the group with both hip and knee OA (Table 1). Total number of comorbidities was highest in the third group of the patients with OA (17, or 8.7%) and at the least in the hip OA group (7, or 3.6%). There were not statistically significant diferences in the presence of comorbidities among three groups ($p > 0.05$).

Table 2 presents differences in QoL and its domains (pain, stiffness and function) among the three groups of patients with various localization of severe hip or knee osteoarthritis, obtained by the Analysis of variance with single factor. Each subscale score was transformed to a range from 0 to 100 points, with a score of 100 indicating no pain, dysfunction, or stiffness. The best QoL was in the group of knee OA (42.7 ± 11.3) and the worst one in the group with both hip and knee OA patients (35.8 ± 12.7). The results showed that there were statistically significant differences among the three groups of OA patients in QoL ($F = 5.377$, $p < 0.01$) and in the domain of physical function ($F = 5.273$, $p < 0.01$).

Table 3 presents the standardized regression coefficients obtained by three multiple linear regression analyses, when adjusted with confounders. QoL (WOMAC scores) was the dependent variables and independent variables were age, BMI, social class, pain, stiffness, physical function and comorbidities (hypertension, cardiomyopathy and Diabetes mellitus). The results have shown that QoL assessed by WOMAC score was statistically significantly associated with pain ($t = 4.424$, $p < 0.001$ and $t = 2.862$, $p < 0.01$) and physical function ($t = 6.839$, $p < 0.001$ and $t = 7.209$, $p < 0.001$) in the first (hip OA) and the second (knee OA) group respectively, whereas in the third group (both hip and knee OA), WOMAC score was statistically significantly associated with BMI ($t = 2.361$, $p < 0.05$), pain ($t = 2.450$, $p < 0.05$), physical function ($t = 9.228$, $p < 0.001$) and Diabetes mellitus ($t = 2.418$, $p < 0.05$).

Discussion

There are reports about risk, prognostic factors, disability, QoL of patients with knee or hip OA and guidelines used for management of hip and knee OA [17–19]. Therapeutic modalities may positively influence pain and function, mobility and QoL in patients suffering from OA of the lower limbs [5]. Possible differences in QoL between hip and knee OA and patients with both hip and knee localization of OA can have impact on planning the therapy for these patients. There are reports about associated increased risk of hip and knee replacement due to osteoarthritis [12, 20, 21] and outcome, QoL and differences of hip and knee OA patients [22, 23]. This cross-sectional study has helped to investigate differences in QoL in patients with severe hip OA, knee OA and with both hip and knee OA. Three multiple linear regression models were used to investigate association of QoL of patients with severe hip or knee OA with potential confounders in all three groups.

Global WOMAC score, which represented the QoL in the OA patients was significantly different ($F = 5.377$, $p < 0.01$) among the three groups of the OA patients (Table 2). Caracciolo and al. [22] also found that differences among the OA groups were evident.

The pain was important for decision whether or not to perform knee and hip surgery [7, 24]. It has significantly influenced QoL in all three groups of the OA patients in our research. Although we did not find that there was statistically significant difference in the domain of pain among patients with these three various localization of severe hip or knee OA.

Table 2

Differences in quality of life and its domains among three groups of patients with various localization of severe hip or knee osteoarthritis (n = 195)

Parameters	Group I (Hip osteoarthritis)	Group II (Knee osteoarthritis)	Group III (Hip and knee osteoarthritis)	F	*p*
WOMAC score	38.9 ± 12.3	42.7 ± 11.3	35.8 ± 12.7	5.377	0.005
Domain					
Pain	9.5 ± 3.7	9.6 ± 3.6	8.7 ± 4.2	1.044	0.354
Stiffness	0.97 ± 1.4	0.65 ± 1.3	0.7 ± 1.4	0.984	0.376
Physical function	27.5 ± 10.1	30.8 ± 8.2	25.4 ± 10.2	5.273	0.006

Note: Values are expressed as mean ($\bar{x}$) ± standard deviation (SD); WOMAC score presented from 0-100, i.e. from worst to best; WOMAC – Western Ontario and McMaster Osteoarthritis Index; F – value of F test statistics (ANOVA).

Table 3

Standardized regression coefficients in three multiple linear regression models of Western Ontario and McMaster Osteoarthritis Index (WOMAC) scores

Independent variables	Group I (Hip osteoarthritis)		Group II (Knee osteoarthritis)		Group III (Hip and knee osteoarthritis)	
	Beta	(t)	Beta	(t)	Beta	(t)
Age	-0.42	0.565 (0.580)	0.030	0.712 (0.371)	0.004	0.960 (0.051)
Body mass index	-0.076	0.308 (1.029)	-0.070	0.379 (0.887)	0.164	0.022 (2.361)
Social class	-0.021	0.781 (0.279)	-0.085	0.322 (0.999)	0.059	0.384 (0.878)
Pain	0.363	0.000 (4.424)	0.236	0.006 (2.862)	0.200	0.017 (2.450)
Stiffness	0.103	0.185 (1.342)	0.164	0.056 (1.949)	0.082	0.270 (1.115)
Physical function	0.573	0.000 (6.839)	0.608	0.000 (7.209)	0.726	0.000 (9.228)
Hypertension	-0.001	0.990 (0.012)	-0.125	0.164 (1.410)	0.043	0.564 (0.580)
Cardiomyopathy	-0.052	0.615 (0.506)	0.55	0.582 (0.554)	0.038	0.603 (0.524)
Diabetes mellitus	-0.012	0.900 (0.126)	0.032	0.751 (0.320)	0.166	0.019 (2.418)

Note: WOMAC score was dependent variable.

Domain of stiffness was not statistically significant different among groups, but physical function was. QoL was statistically significantly associated with physical function in all three groups of OA patients. The group with knee localization of OA had the highest score of physical function. These results are in accordance with other reports [12, 25].

Average age of the knee OA patients placed on the waiting list for total joint replacement was higher than in hip OA patients which is in accordance with other reports [25, 26], but there was no significant association between QoL and age in any of the three groups of the OA patients. The least average age was recorded in the third group with both hip and knee OA. Difference among the three groups of the patients was not statistically significant in our research. These results may be explained by the fact that function in the knee OA patients was better than in the hip OA patients and the patients with knee OA may have made a decision for arthroplasty later than patients with hip OA and patients with both hip and knee OA. The third group of the patients included both hip and knee OA, which could have influenced such result (the least average age).

Females had higher percentage of OA than males in our study. There was statistically significantly higher number of females than males in all three groups of the OA patients. These results are in accordance with findings that the prevalence of osteoarthritis-related disability is greater among women than among men [13]. Maillefert et al. [13] state that hip OA in women is more frequently part of polyarticular OA and displays greater symptomatic and structural severity. Severe hip and knee OA were more often on the right (55.8%) than on the left (44.2%) side in patients with OA in our research. But, we did not find statistically significant difference among the groups with hip, knee and both (hip and knee) localization in relation to side of the OA.

Comorbidities (hypertension, cardiomyopathy, diabetes mellitus) were not often in any of the three groups of the OA patients in our research (17.9%). This is in accordance with other findings [13]. Tuominen and al. [21] reported that incidence of comorbidity in their investigation was 73%. These results may be explained by the fact that comorbidities included in these two investigations were different. But, QoL was significantly associated with Diabetes mellitus in the group of patients with both hip and knee OA in our research. This is in accordance with recently published systematic literature review and meta-analysis study. It has shown an association of diabetes mellitus and OA, but causality is not yet clearly demonstrated [27].

All three groups of patients in our research were in range of obesitas, but differences were not statistically significant. QoL of the OA patients was significantly associated with BMI only in the group of patients with both hip and knee OA. Recent study indicates that obese patients were more present and underwent joint replacement surgery at a younger age as compared to nonobese patients [28]. Women in the highest category of BMI had a twofold increased risk of hip replacement due to OA [26], and BMI together with age and gender influence the decision to perform knee replacement surgery [24]. Arthritis was associated with an increasing negative impact on health and QoL for older women over time [29].

Social class did not significantly influence QoL of the patients with OA with different localization of OA in our research. Differences in relation to other reports may be due to different definition of social impact, design of the investigation and different social and country status as well as insurance.

QoL, pain and physical function were the worst in the group of OA with both hip and knee OA. The pain and physical function were significantly associated with the QoL in patients with all three localization of OA and could be the most important factors in making decision for arthroplasty. Also, the QoL of patients with both the hip and knee OA was signifficantly associated with BMI and diabetes mellitus. These findings are in accordance with other reports [19, 24, 25, 27].

OA hip and knee are often associated with significant pain, disability, and impaired QoL. The effect of size of a specific treatment might vary according to the site of the OA involvement owing to differences in anatomy, biomechanics, risk factors for development and progression, accessibility to local treatments and other factors [30]. Understanding what we know (and do not know) about hip, knee and OA with both, hip and knee localization differences is critical for improving quality of care for our patients and findings in this study could be useful in practice and in further investigations. These factors are the strengths of this study. The primary limitation of the study is that we could not include as confounders emotional domains and that we used only disease-specific instrument for measurement of QoL. Further studies are required to confirm our results in other sets of patients and better understand the underlying mechanisms of differences among the hip, the knee and both the hip and knee OA.

Conclusion

Our results show that there are differences between patients with severe hip, knee and both hip and knee osteoarthritis in QoL with the lowest WOMAC score in the group of the patients with both hip and knee osteoarthritis and the highest score in the knee osteoarthritis group of the patients. Domain of physical function was statistically significantly different among groups of osteoarthritis patients with the highest score in knee osteoarthritis group of the patients. The pain and physical function were significantly associated with QoL in the severe hip and knee OA patients. QoL in the group with both hip and knee OA patients was significantly associated with the pain, physical function, BMI and diabetes mellitus.

These findings are important and could be useful in practice and in further investigations for improving quality of care of osteoarthritis patients, especially in pain and function domains. They can help in considering the treatment of osteoarthritis patients and in decision of the time of surgery.

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Finger and foot tapping sensor system for objective motor assessment

Senzorski sistem za objektivnu motornu procenu na osnovu *tapping*-a prstima i stopalom

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Abstract

Background/Aim. Finger tapping test is commonly used in neurological examinations as a test of motor performance. The new system comprising inertial and force sensors and custom proprietary software was developed for quantitative estimation and assessment of finger and foot tapping tests. The aim of this system was to provide diagnosis support and objective assessment of motor function. **Methods.** Miniature inertial sensors were placed on fingertips and used for measuring finger movements. A force sensor was placed on the fingertip of one finger, in order to measure the force during tapping. For foot tapping assessment, an inertial sensor was mounted on the subject's foot, which was placed above a force platform. By using this system, various parameters such as a number of taps, tapping duration, rhythm, open and close speed, the applied force and tapping angle, can be extracted for detailed analysis of a patient's motor performance. The system was tested on 13 patients with Parkinson's disease and 14 healthy controls. **Results.** The system allowed easy measurement of listed parameters, and additional graphical representation showed quantitative differences in these parameters between neurological patient and healthy subjects. **Conclusion.** The novel system for finger and foot tapping test is compact, simple to use and efficiently collects patient data. Parameters measured in patients can be compared to those measured in healthy subjects, or among groups of patients, or used to monitor progress of the disease, or therapy effects. Created data and scores could be used together with the scores from clinical tests, providing the possibility for better insight into the diagnosis.

Key words:
parkinson disease; muscle tonus; neurophysiology; toe phalanges; hand; fingers; equpment and supplies; serbia.

Apstrakt

Uvod/Cilj. *Tapping* tj. tapkanje prstiju šake i stopala se uobičajeno koristi u neurološkim ispitivanjima kao test motorike. Prikazan je novi sistem koji sadrži inercijalne senzore i senzore sile, kao i odgovarajući softver za kvantitativnu procenu dijagnostičkog motornog testa na osnovu *tapping*-a prstima i stopalima. Uz pomoć ovog sistema moguća je objektivna evaluacija motornog obrasca bolesnika, a samim tim i lakše postavljanje određenih dijagnoza i praćenje progresa bolesti ili terapije. **Metode.** Minijaturni inercijalni senzori su bili postavljeni na vrhove prstiju u cilju kvantifikovanja pokreta prstiju. Senzor sile postavljen je na jagodicu jednog prsta i merio je silu primenjenu u toku *tapping*-a – tapkanja kažiprsta o palac. Za ocenu *tapping*-a stopalom, inercijalni senzor je postavljen na gornji deo stopala ispitanika koje je bilo postavljeno na platformu za merenje sile. Pomoću ovog sistema mogu se posmatrati brojni parametri poput broja i trajanja svakog pokreta, ritma i promena ritma, brzine otvaranja i brzine zatvaranja prstiju, primenjene sile, promene ugla između prstiju, i na osnovu ovih parametara može se vršiti detaljna analiza motornog stanja bolesnika. Sistem je testiran na 13 bolesnika sa Parkinsonovom bolešću i 14 zdravih ispitanika. **Rezultati.** Sistem je omogućio jednostavno merenje navedenih parametara i grafički prikaz kvantitativnih razlika u ovim parametrima između zdravih ispitanika i bolesnika sa neurološkim oboljenjem. **Zaključak.** Novi sistem za *tapping* prstima i stopalima je kompaktan, jednostavan za upotrebu i efikasan za prikupljanje podataka o bolesniku. Izmereni parametri mogu se koristi za poređenje bolesnika sa zdravim ispitanicima, ili sa drugim grupama bolesnika, ali i za praćenje progresa bolesti ili efekata terapije. Dobijeni podaci mogu se koristiti zajedno sa rezultatima drugih kliničkih testova, dajući tako mogućnost za bolji uvid u dijagnozu.

Ključne reči:
parkinsonova bolest; mišići, tonus; neurofiziologija; prsti noge; šaka; prsti; oprema i pribor; srbija.

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Introduction

Finger tapping test is commonly used in neurological examinations as the test of motor performance [1]. Patients tap their thumb and index fingers as quickly as possible for a required period of time, usually 10 to 15 s. The rhythm, amplitude, and velocity of tap movements depend on patient's motor capabilities and symptoms, providing an estimation of the integrity of central nervous system components [2]. Foot tapping is proven to be a reliable and valid measure of Parkinson's disease (PD) motor function [3] and estimation of rigidity or tremor in PD [4].

Large differences between the performance of the fingers on the left and right hand or differences in left and right foot speed may reflect a lateralized hemispheric dysfunction. Holmes [5] already proved that the rhythm of finger tapping movements acts as an efficient index for cerebellar function testing. Tapping tests have been widely used for quantification of ataxia [6], assessment of stroke recovery [7] or quantification of Alzheimer's disease [8].

Repetitive finger tapping is commonly used to assess bradykinesia in Parkinson's disease. It is included in the Unified Parkinson's Disease Rating Scale (UPDRS test, e.g. Fahn et al. [9] 1987), providing descriptive characteristics of the patient motor ability. UPDRS levels are categorized as: mild slowing and/or reduction in amplitude; moderately impaired; severely impaired, with frequent hesitation in initiating movement or arrests in ongoing movement, and, can barely perform the task. In patients with PD, finger tapping was selected as it is more severely affected than hand opening and closing, and hand pronation and supination elements of the motor section of Part III of the UPDRS [10, 11]. The rhythm, amplitude and velocity of the index tap movements vary with patient's motor capabilities and symptoms. Tapping is simple and commonly used in assessment, and any distinctive features identified for the condition would provide helpful diagnostic clinical clues. Furthermore, the foot tapping technique was used to compare reliability to measure improvement in parkinsonism during different applied medication [3]. It has been shown that foot tapping provides more information than finger tapping, i.e., the alternate foot tapping correlates better with PD outcome measures than finger tapping [3]. Foot tapping may be a useful outcome measure for determination of dopaminergic medication effect in PD clinical trials [4].

In clinical practice tapping is often evaluated visually, estimating speed and regularity of the movements. However, very small finger tapping differences in amplitudes cannot be easily and correctly identified during neurological examination. It has already been reported that tapping score is one of the most difficult items to assess [12]. Several tapping-measuring mobile devices were described in literature, [13] with different measurement protocols – finger tapping, alternate and repetitive foot-tapping (between two, or on one pedal) [3]. Their aim was to create an efficient system for use in general clinical environment and to validate the measurement and evaluation method for finger and foot tapping movements.

Several research groups have worked on making quantitative evaluation more accurate through the use of various finger-tapping systems. Some relied on 3D recordings from an optoelectronic motion capture system with markers placed on a hand of a subject for reconstructing the tapping motions [14–16]. In other studies, different kind of sensors were mounted on a subjects' fingers, or were constructed in form of touch pads [17–20].

Okuno et al. [17] presented a finger tapping acceleration measurement system for the quantitative diagnosis of PD, which uses 3-axis piezoelectric accelerometers, a pair of touch sensors made of thin stainless steel sheets, an analog to digital converter and a personal computer. Finger stalls, with these sensors, were attached to the index finger and thumb, and the subjects were prompted to perform finger tapping motion, so that their index finger and thumb should touch, continuously for 60 s at a time. They showed that relevant features could be extracted from accelerometer and touch sensor output. The features included standard deviation of single finger-tapping intervals, average of maximum single finger-tapping velocities and average of maximum single finger-tapping amplitudes.

Ling et al. [14] observed the same type of movement with a measurement system that consisted of infrared emitting diodes placed across the subject's hands and a 3D motion analyzer. Amplitude, cycle duration and mean speed were measured for each cycle of finger tapping from one finger-thumb separation to the next. This study showed a difference in tapping patterns between patients with PD and those with progressive supranuclear palsy.

A study using an image based motion analyzer was introduced by Jobbágya et al. [15] who analyzed motion of fingers while simulating playing the piano. They assessed the speed and regularity of these movements in patients with PD and a control group of healthy subjects, with the help of an image based motion analyzer and passive markers attached to anatomical landmark points. Another group [18] developed a system for estimating piano-playing-like motions, designed in form of four electronic touch plates in fan shape and a hand rest as the fifth plate. An oscillator was attached to the fifth plate, which resistively induced a small sinusoidal current in the hand. When a finger should touch one of the touch plates, the induced signal on the finger would be of sufficient amplitude to toggle the output of a digital logic gate. Subjects had their free tapping motion tested, as well as tapping with weights attached.

An interesting system was presented by Shima et al. [21], working with a magnetic sensor with two coils mounted on the hand of a subject. The coil voltage created by electromagnetic induction changed depending on the distance between the two coils. The system had a graphical output, displaying the measured fingertip distance, velocity, acceleration, computed indices and radar charts, phase-plane trajectories of the fingertip distance and velocity, as well as velocity and acceleration in real-time.

Despite various mentioned and other related systems, currently there is no commercially available system for finger and foot tapping assessment in patients with PD or rela-

ted movement disorders. In this paper, we propose a novel sensor system for quantitative and qualitative finger and foot tapping assessment. The system comprises miniature inertial sensors placed on the index and thumb finger ends (top side), or on the upper side of the foot. Along with inertial sensors, the system includes a force sensor placed on a fingertip and a force platform for foot tapping force assessment. The system outputs are quantitative measures, such as tapping durations, number of taps, tapping velocity, tapping force, and tapping angle (angle between the fingers or between the foot and the ground). The system was used to record tapping in neurologic patients as well as in healthy controls.

Methods

Instrumentation

The system comprises of three sensor control units (SCU) which acquire signal data from the sensors and wirelessly transmit them to a remote computer though the interface unit (Figure 1). Data acquisition is controlled through a user-friendly graphical interface. Wireless communication enables convenient usage of the system in clinical environment, covering the radius of 20 m indoors [22].

Both the index finger and the thumb are mounted with sensors and connected to their SCU (SCU1 and SCU2 in Figure 1). In order to measure the contact force between the fingers, SCU1 is additionally equipped with a force sensing resistor (FSR, Interlink, USA), connected to the control unit with a tiny cable. The third control unit (SCU3), used for foot tapping, is additionally connected to a force sensing platform. The force platform is a custom made combination of active (metatarsal) and passive (heel) areas. The mechanical construction of the platform enables free movement of the active plate in the nominal force range up to 50N, while the passive plate is connected to the fixed part of the platform. A load cell (AMI-5, GLIKI, Austria) is placed between active and passive metal plates, so it measures the force applied to the active area. The load cell interface contains an instrumentation amplifier and additional passive electronic components. The gravitational component is eliminated by software calibration (Figure 2).

Data is sampled with 200 samples per second. The effective resolution is 12 bits for the inertial sensors, while for the force sensing sensors the effective resolution is 8 bits. The acquired signals are monitored online and automatically stored for further processing. The acquisition software was designed in LabWindows CVI (National Instruments, USA),

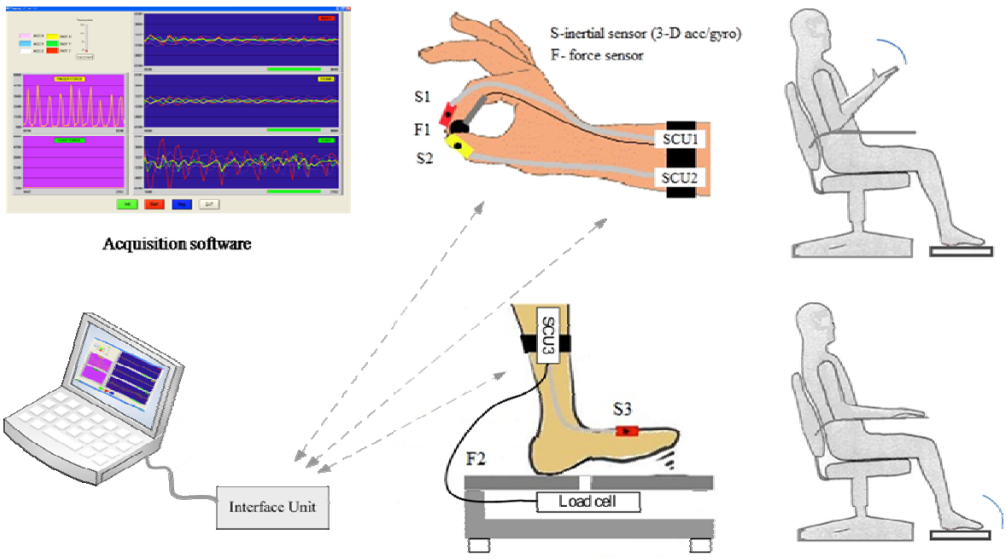

Fig. 1 – Left: Finger and foot tapping acquisition scheme. Middle panels: placements of the inertial sensors (Si) and force sensors (Fi) for finger (Top) and foot (Bottom) tapping. Right panels: initial subject's position during finger (Top) and foot (Bottom) tapping testing.

Each SCU is equipped with a miniature inertial measurement unit (IMU), which comprises of a 3D accelerometer LIS3DH, and a 3D gyroscope L3G4200 (STMicroelectronics, USA). IMUs and control units are connected with a tiny flat cable. IMUs are placed directly either on the finger or foot, while the control unit is attached to the stable part of the body in the vicinity (arm or leg, respectively). IMU's are light, with small dimensions, allowing the subject to perform the movements in a natural manner.

while signal analysis was performed in Matlab (MatWorksInc, USA).

Participants

This study included two groups of right-handed participants: 13 patients with PD diagnosed according to the UK Queen Square Brain Bank Criteria [23]; 14 healthy controls (CTRL) with no history of neurological or psychiatric disea-

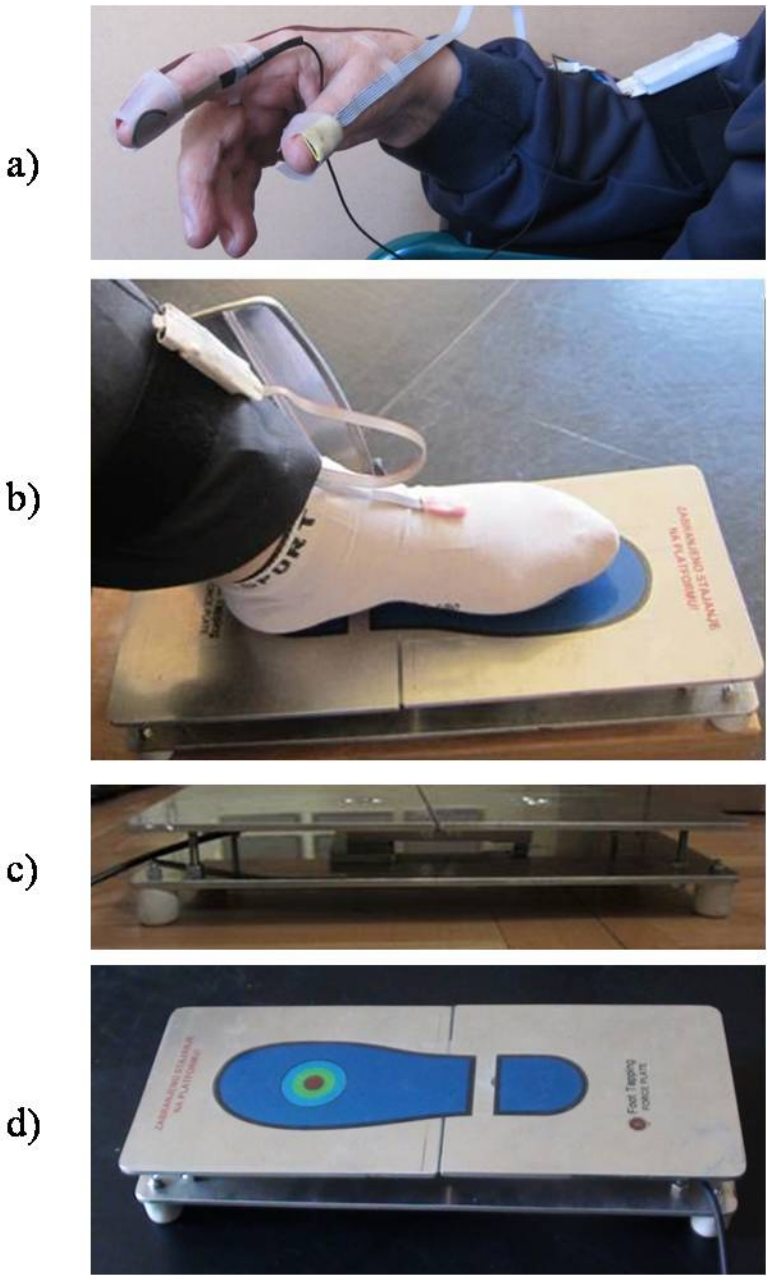

Fig. 2 – Sensor system for finger and foot tapping: a) finger tapping system mounted on a patient; b) foot tapping system mounted on a patient; c) force platform (cross section); d) force platform (view from above).

se. CTRLs were age- and sex-matched with the patient group (Table 1). Participants were recruited from the Movement Disorders Unit at the Clinic for Neurology, Clinical Centre of Serbia, Belgrade.

Patients with tremor/dyskinesia and hand dystonia, as well as any disability of the extremities that might interfere with motor tasks, were excluded from the study. Other exclusion criteria were: scores < 26 on the Mini Mental Status Examination [24] and < 15 on the Frontal Assessment Battery [25], respectively; score > 14 for the Hamilton Depression Rating Scale [26]; and history of psychosis or major medical disease.

Disease staging was assessed according to the Hoehn and Yahr [27] system and motor disability using the UPDRS III [9]. Levodopa equivalent dose was also calculated [28]. All the tests, including FT performed in accordance with the recommendations for FT assessment, were conducted in the morning after an overnight treatment withdrawal of at least 12 hours where applicable (patients with PD were tested during "off" time) [19].

Experiments: system setup and recording protocol

Subjects were asked to sit comfortably in a chair. The sensors were carefully mounted on patients' fingers so as to minimize obstruction of natural movements. The inertial sensors (S_i) were placed on top of index and thumb nails, along the finger's length, while the force sensor (F_i) was placed on finger tip (Figure 1, upper middle panel). The sensors were fixed with Leucopor® or similar adhesive tape. Complete

Table 1
Demographic and clinical features of patients with Parkinson's disease (PD) and healthy controls (CTRL)

Parameters	CTRL (n=14)	PD (n=13)	*p* value
Age (years)	56.8 ± 9.0	60.9 ± 9.9	/
Female/Male	8/6	6/7	/
Disease duration, years	/	4.6 ± 4.5	/
LED (mg/day)	/	664 ± 531	/
Hoehn&Yahr Stage	/	2.1 ± 0.9	/
UPDRS total	/	47.1 ± 18.9	/
UPDRS motor part	/	27.2 ± 10.3	/
MMSE	29.4 ± 0.9	28.8 ± 1.1	0.001
HDRS	4.0 ± 2.1	8.2 ± 4.7	0.023
FAB	17.9 ± 0.3	15.5 ± 1.3	0.001

Note: Values present mean ± standard deviation.
HDRS – Hamilton Depression Rating Scale; LED – levodopa equivalent dose; UPDRS – Unified Parkinson's Disease Rating Scale; MMSE – Mini Mental Status Examination; FAB – Frontal Assessment Battery.

mounting of the sensors and system setup requires less than five minutes.

For the finger tapping test, the subjects were asked to place their hand in front of them in the way they found most convenient (Figure 1, top right). In order to allow unobstructed foot tapping, the chair height was carefully adjusted so that the subject's thighs were parallel to the ground, knee's flexion less than 90 deg, and there was enough distance between the seat borderand the knee (Figure 1, bottom right).

Before the tapping, the participant's maximal voluntary contraction (MVC) was recorded. The participants were asked to press the sensor between their index and thumb fingers as hard as they can for 5 s, or in the same manner, to press the force platform with their metatarsal and toe areas. After that, the participants were instructed to repeatedly tap their index finger and thumb as rapidly and as widely as possible for 15 s [14]. The same time period was recorded for repetitive foot tapping, using a single pedal. Because fatigue may affect performance, a rest period of one minute is given between trials. Each trial began and ended with fingers closed, or foot placed on the force platform (zero angle). Both hands and both feet were tested.

The recordings of subjects and different patient groups were performed at the Clinic for Neurology, Clinical Centre of Serbia, Belgrade. The study was performed in accordance with the ethical standards of the Declaration of Helsinki. All participants gave written informed consent prior to participation in the study.

Signal processing

In order to provide 3-D movement analysis, we estimated the angles between the index finger and the thumb (finger tapping angle). The developed software employs transformation matrices and introduces biomechanical constraints of tapping movements [22]. Hand orientation or possible changes in position and orientation are irrelevant for the system performance. Tapping segmentation is performed based on estimated angles through identification of local maxima/minima. This segmentation is additionally confirmed from force sensors by applying threshold clipping to 5% of their values normalized to its maxima. This kind of normalization is applied only for tapping segmentation. Forces which are displayed as system output are normalized to MVC, i.e., normalized to the maximal force between the fingers applied on the force sensor (Fi) and maximal force applied by metatarsal and toe area on the force platform. Tapping speed is estimated as a derivative of the tapping angle.

Results

The recorded data were extracted from the storage medium and analyzed.

First, we presented examples from one healthy subject and one patient with a neurodegenerative disease manifested with movement disorders (Figures 3–6). Extracted and analyzed data were displayed on the computer screen or printed and added to a patient's chart. Obtained results allowed clinicians to monitor movements of the fingers and foot during tapping.Tapping performance may be followed through the series of quantitative parameters (Figures 4 and 6) such as duration of each tap, tapping frequency, "open" and "close" speed for finger tapping (i.e., "upward" and "downward" speed for foot tapping), and by monitoring the force and tapping angles achieved during tapping (Figures 3 and 5). Visual inspection of presented results clearly pointed out the difference between the patient and the healthy control subject.

Here we present the results for the tested groups of PD patients and healthy controls. Group results for patients with PD and healthy controls are presented in Figures 7 and 8, for finger and foot tapping, respectively.

The upper panels show calculated mean values for the tapping amplitude (angle), tapping duration and tapping speed. The results are presented with bar charts presenting average values with standard deviations within the observed group.

Progressive changes in amplitude, duration and speed across a 15 s tapping trial can be represented by the slope of

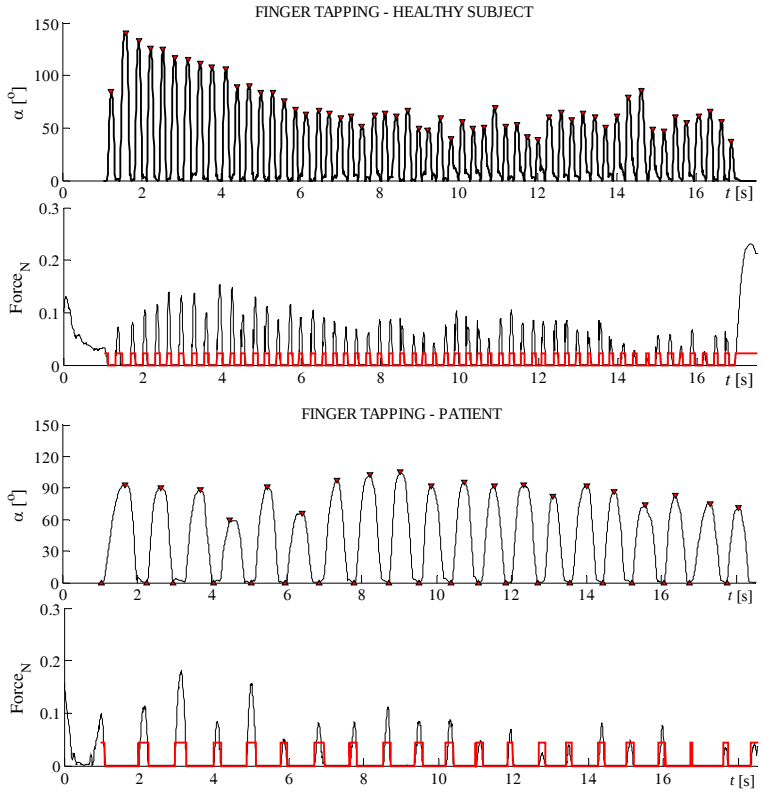

Fig. 3 – Estimated finger tapping angle and measured force normalized to maximal voluntary contraction (MVC), example for one healthy subject and one patient. The duration of finger tapping contacts are marked with red rectangular pulses over force traces. Maximal tapping angles (fingers "open") are marked with red triangular markers pointing downwards. Minimal tapping angles (fingers "closed") are marked with triangular markers pointing upwards, and they are used as separator of consecutive taps.

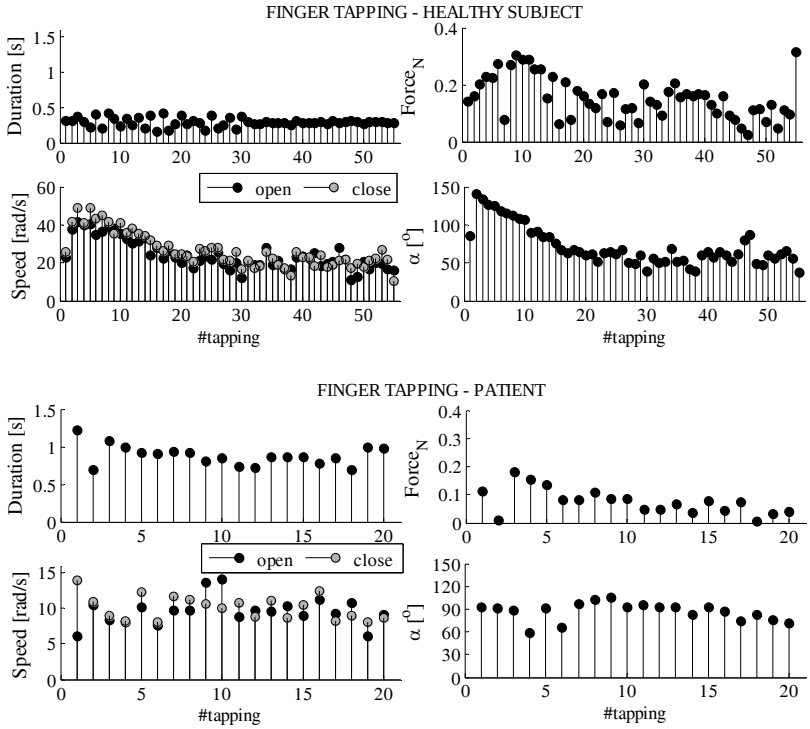

Fig. 4 – Finger tapping parameters: tapping duration, speed, normalized force and tapping angle, example for one healthy subject (upper four panels) and one patient (lower four panels). Horizontal axes show the order of taps.

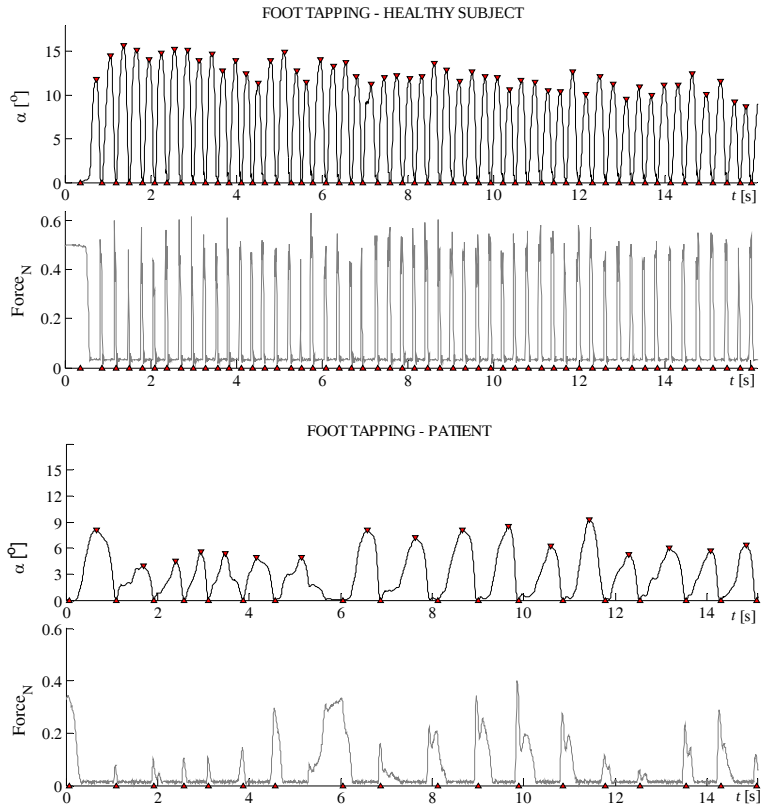

Fig. 5 – Evaluated foot tapping angle and normalized force (upper and lower panel, respectively). Triangular markers oriented upwards separate taps. Triangular markers oriented downwards (upper panel) show the maximal angle achieved within the particular tap (upper two panels – healthy subject: lower two panels – patient).

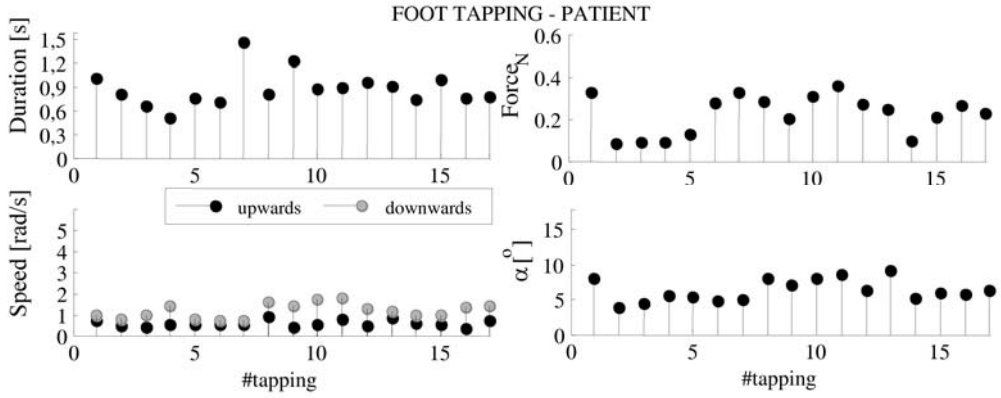

Fig. 6 – Foot tapping parameters: tapping duration, speed (separately for upward/downward foot movements), normalized force, tapping angle.
Upper four panels: example for one healthy subject; lower four panels: example for one patient.

the fitted linear regression line. The slope of change in amplitude can be used to assess progressive hypokinesia or "decrement". The slope of change in speed that encompasses both amplitude and duration can be used to assess progressive slowing of movement[14]. The slopes of finger and foot tapping movements for the observed kinematic parameters are also presented in Figures 7 and 8, in lower rows.

The numerical results for the performed tapping testing are shown in Table 2. The Table also presents the coefficients of variation (CV) of amplitude and speed across the tap trials [29].

Discussion

We emphasize several important aspects of the system presented here.

The system is easy to mount and allows recording of finger and foot tapping even in patients with very limited

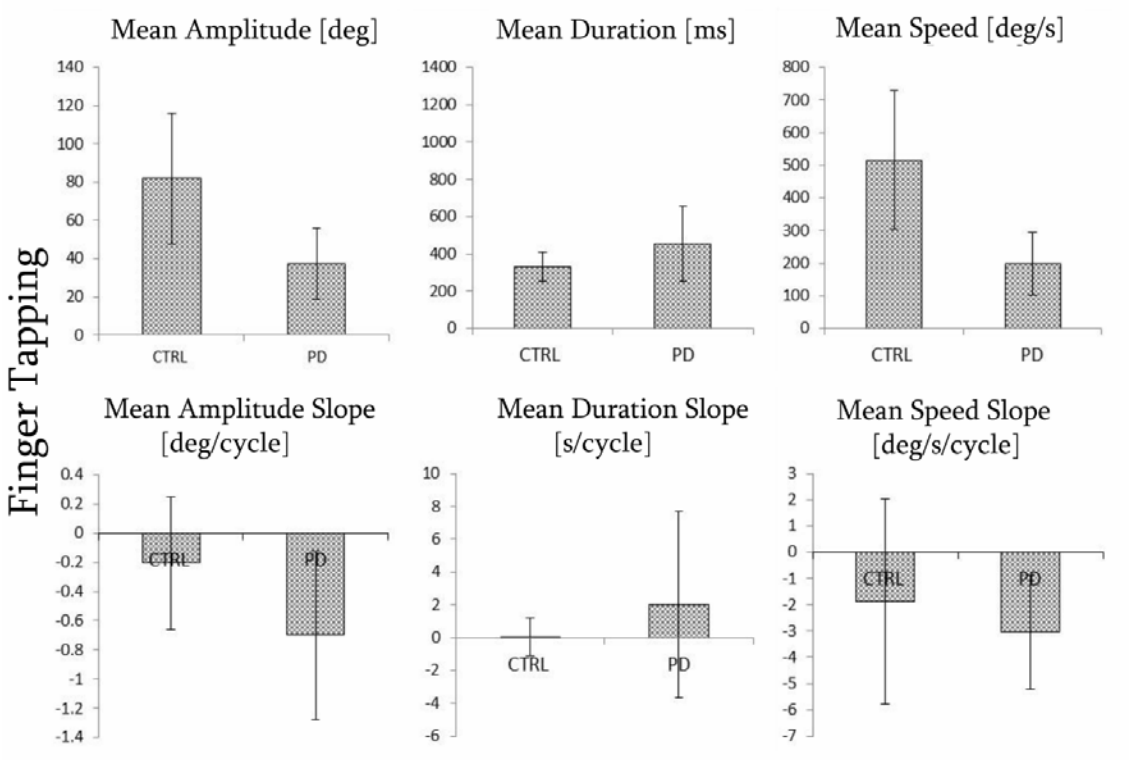

Fig. 7 – Kinematic finger tapping parameters (amplitude-left panel, duration – middle panel, and speed – right panel) of patients with Parkinson's disease (PD) and healthy controls (CTRL).
Parameters are presented according to their mean (upper row) and slope (lower row) values.
Each bar shows average values with standard deviations.

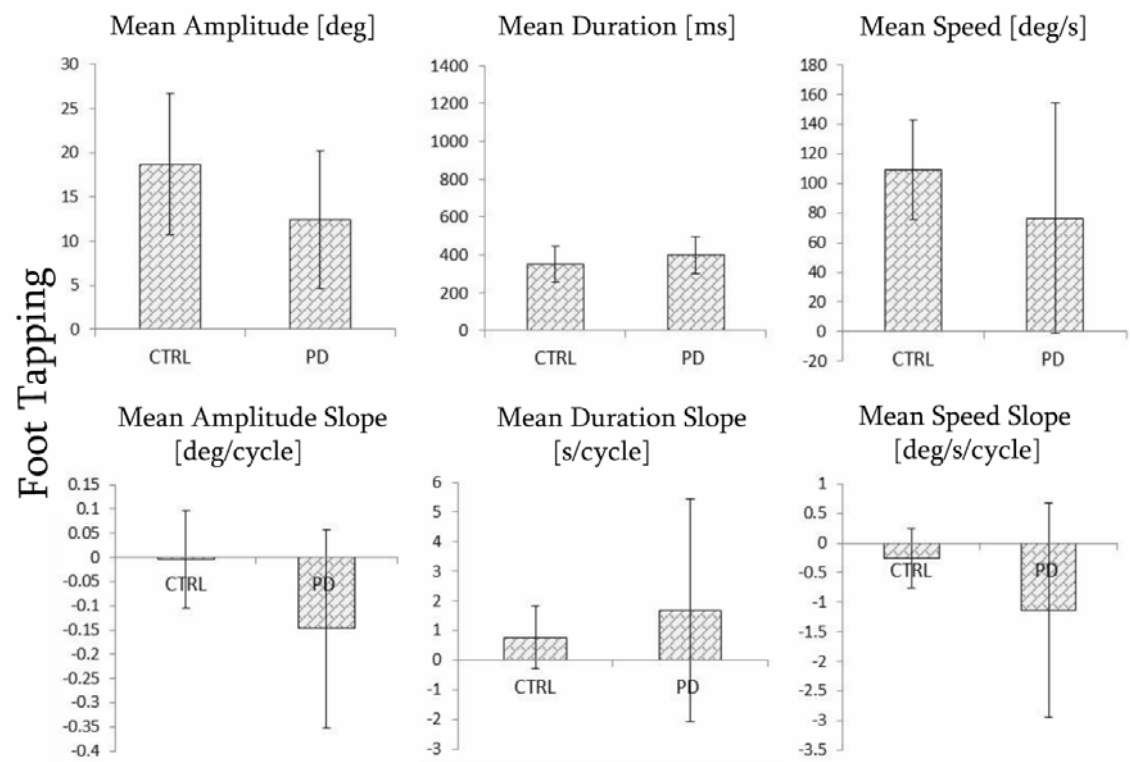

Fig. 8 – Kinematic foot tapping parameters (amplitude –left panel, duration - middle panel, and speed - right panel) of patients with Parkinson's disease (PD) and healthy controls (CTRL).
Parameters are presented according to their mean (upper row) and slope (lower row) values.
Each bar shows average values with standard deviations.

Table 2

Analysis of kinematic parameters during finger tapping task

Parameters	Finger tapping		Foot tapping	
	CTRL (n = 14)	PD (n = 13)	CTRL (n = 14)	PD (n = 13)
Cadence [n/15s]	47,81 ± 12.65	40,11 ± 18,37	46.61 ± 12.78	41.18 ± 12.45
Amplitude [deg]	81.82 ± 33,94	37,18 ± 18,50	18.76 ± 8.11	12.53 ± 7.86
Duration [ms]	331.74 ± 76.79	454.33 ± 201.86	344.4 ± 92.31	388.85 ± 91.52
Close velocity [deg/s]	-1602,7 ± 503,1	-676,4 ± 370,5	-306.35 ± 167.7	-241.46 ± 175.37
Open velocity [deg/s]	1148,08 ± 499,05	483,52 ± 236,65	211.05 ± 82.93	157.933 ± 57.73
Speed [deg/s]	516,58 ± 213,88	198,25 ± 96,34	109.57 ± 33.38	76.7 ± 37.76
Amplitude CV [%]	12,31 ± 5,44	35,52 ± 14,15	12.09 ± 4.22	22.99 ± 12.39
Duration CV [%]	14,48 ± 6,93	22,79 ± 6,34	9.41 ± 6.14	15.03 ± 8.02
Speed CV [%]	16,14 ± 6,66	33,67 ± 12,31	12.45 ± 4.25	26.18 ± 12.19
Amplitude slope [deg/cycle]	-0,21 ± 0,46	-0,70 ± 0,58	0.0025 ± 0.11	-0.12 ± 0.21
Duration slope [ms/cycle]	0,04 ± 0.001	2,021 ± 5,67	0.51 ± 0.68	1.44 ± 0.81
Speed slope [deg/s/cycle]	-1,88 ± 3,89	-3,04 ± 2,18	-0.22 ± 0.52	-1.06 ± 1.8

Values present mean ± standard deviation; PD – Parkinson's disease; CTRL – healthy controls; CV – coefficient of variation.

movements. The sensors are lightweight and miniature, and do not hinder patient's movements. Also, the sensor do not require careful positioning, they just need to be placed on top of fingers (or foot), and the auto-calibration procedure will set the axes for further calculations. This is particularly important since it means that the system does not need specially trained medical or technical staff. The benefits of the proposed systems also include the economical aspect. The proposed system is low cost compared to any other commercially available system for motion capture. Using inertial sensors and force platform, any clinic could afford to introduce such system and methodology in their assessments.

The system is used for objective evaluation of the patients, as an addition to standard clinical tests and scoring system. It provides quantitative assessment, which is stored in database, and can be compared to the patient's previous recordings, thereby monitoring progress of the disease, or response to therapy. After recording, the software enables analysis of tapping sequence, and it displays the recorded sequence. It also enables observing the numerical results, offering list of parameters. The recorded data can be studied in two ways: by analyzing the numerical values of kinematic parameters – the average performance for the specified parameters, coefficients of variations, trends of changes, minima and maxima etc.; by observing the shapes of kinematic parameters – identifying problems with tapping rhythmicity, regularity, smoothness, freezing, tremor, and other irregular events that could be present in their motor pattern.

The proposed system also supports comparison among patients, or patients with healthy subjects, therefore providing a significant tool for studying characteristics of different epidemiologies [30].

The obtained data and numerical results could be used together with scores from clinical tests, providing better insight into the diagnosis. Future research efforts will be directed at upgrading the system software to an expert system that would further assist clinicians in diagnostic procedures. A large number of particular patient groups would provide referent values for specific parameters, such as frequency, velocity, developed force and angles between fingers. This would enable automatic diagnostic indication in different groups of patients.

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Conclusion

The novel system for finger and foot tapping test is compact, simple to use and efficiently collects patient data. Parameters measured in patients can be compared to those measured in healthy subjects, or among groups of patients, or used to monitor progress of the disease, or therapy effects. Created data and scores could be used together with the scores from clinical tests, providing the possibility for better insight into the diagnosis.

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R E F E R E N C E S

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Retrospective analysis of 1,211 operated patients due to groin hernia with open surgical approach – single center experience

Retrospektivna analiza 1 211 operisanih bolesnika zbog ingvinalne kile otvorenim hirurškim pristupom – iskustvo jednog centra

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Abstract

Bacground/Aim. Groin hernias are common pathology among men population. Only curative treatment is surgical reparation with various surgical procedures for groin hernia solving. The aim of this study was to evaluate the most prevalent surgical procedures and early postoperative complications after groin hernia reparation in large series of operated patients, and to assess the morphologic characteristics of groin hernias. **Methods.** The retrospective study included all patients with groin hernia who underwent surgical reparation from 2009 to 2012. In all patients a demographic characteristics, including gender and age, clinical characteristics and hernia type were analyzed. The surgical procedure for hernia solving and early postoperative complications were assessed. **Results.** The study included 1,211 patients. The male/female ratio was 1,127/84 ($p < 0.001$). Inguinal hernia was found in 1,195 patients (94.5% males). Femoral hernia was found in 16 patients (25% males and 75% females). Significant difference in distribution of inguinal and femoral hernia between genders was found ($p < 0.001$). In males right sided inguinal hernia was present in 57.6%. In females right sided inguinal hernia was present in 7 and left sided in 5 patients. Sixsten patients had bilateral inguinal hernia, all in males. There was no significant difference in side of inguinal hernia occurrence and gender. Right sided and left sided femoral hernias were present in the same percent in males. In females a higher occurrence in femoral hernia was found on the right side then on the left one (7:5) without significant difference. There were 71.1% of patients in the age group of 51–80 and 27.2% of patients in the age group of 61–70. Surgical procedures included: Lichtenstein in 51.2% of patients, nylon-darn in 29.6% of patients, Bassini in 16.2% of patients, Lothaissen in 1.7% of patients, and Halsted in 1.4% of patients. Overall, postoperative complications were present in 78 (6.4%) of patients. Wound infection was the most common complication, occurred in 2.4% of patients. **Conclusion.** Prevalence of inguinal hernias is higher in men population, while femoral hernias are more common in females. The most affected population is at the age between 61 and 80 years. The most commonly used open surgical procedures for groin hernia reparation are Lichtenstein and nylon-darn. Both methods have low and similar incidence rates of postoperative complications.

Key words:
hernia, inguinal; hernia, femoral; men; women; age of onset; incidence; surgical procedures, operative; postoperative complications.

Apstrakt

Uvod/Cilj. Preponske kile su česte u muškoj populaciji. Jedini način lečenja preponskih kila je hirurški sa različitim hirurškim procedurama. Ciljevi ovog istraživanja bila je analiza najčešćih hirurških procedura u rešavanju preponskih kila i ranih postoperativnih komplikacija, kao i ispitivanje morfoloških karakteristika preponskih kila kod velikog broja operisanih bolesnika. **Metode.** Retrospektivna studija obuhvatila je sve bolesnike operisane zbog preponske kile u periodu od 2009. do 2012. godine. Kod svih bolesnika analizirane su demografske karakteristike uključujući pol i godine, kao i kliničke karakteristike i tip kile. Analizirane su hirurške procedure u rešavanju kile, kao i rane postoperativne komplikacije. **Rezultati.** Studija je obuvatila 1 211 bolesnika. Odnos muškarci/žene bio je 1,127/84 ($p < 0.001$). Ingvinalna kila bila je prisutna kod 1 195 bolesnika (94,5% su bili muškog pola). Femoralna kila bila je prisutna kod 16 bolesnika (25% muškarci i 75% žene). Nađena je statistički značajna razlika u distribuciji ingvinalne i femoralne kile između polova ($p < 0.001$). Desnostrana ingvinalna kila bila je prisutna kod 57,6% bolesnika muškog pola. Kod žena desnostrana ingvinalna kila bila je prisutna kod 7, a levostrana kod 5 bolesnica. Obostranu ingvinalnu

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kilu imalo je 16 bolesnika, svi su bili muškog pola. Nije bilo statistički značajne razlike u lokalizaciji ingvinalne kile i pola. Desnostrane i levostrane femoralne kile bile su prisutne u istom procentu kod osoba muškog pola. Kod žena veća učestalost femoralne kile bila je sa desne strane (7:5), bez statistički značajne razlike. U starosnoj grupi između 51–80 godina bilo je 71,1% bolesnika, a 27,2% bolesnika u starosnoj grupi od 61–70 godina. Metode hirurškog lečenja obuhvatile su: Lichtenstein kod 51,2% bolesnika, nylon-darn kod 29,6% bolesnika, Bassini kod 16,2% bolesnika, Lothaissen kod 1,7% bolesnika i Halsted kod 1,4% bolesnika. Ukupno, postoperativne komplikacije bile su prisutne kod 78 (6,4%) bolesnika. Infekcija rane bila je najčešća komplikacija, prisutna kod 2,4% bolesnika. **Zaključak.** Zastupljenost ingvinalne kile je veća u muškoj, dok je femoralna kila češća u ženskoj populaciji. Najčešće javljanje preponske kile je u populaciji između 61 i 80 godina starosti. Najčešće korišćene metode otvorenog hirurškog lečenja preponske kile obuhvataju Lichtenstein i nylon-darn procedure. Obe metode imaju malu i sličnu učestalost postoperativnih komplikacija.

Ključne reči:
hernija, ingvinalna; hernija, femoralna; muškarci; žene; životno doba; incidenca; hirurgija, operativne procedure; postoperativne komplikacije.

Introduction

A hernia is defined as a defect of the anterior muscle-aponeurotic and fascial abdominal layer continuity, respiratory or pelvic diaphragm, which permits the protrusion of any tissue, apart those which have normal protrusion throughout the openings in anterior abdominal wall [1]. The etiology of groin hernias involved hereditary and acquired factors, such as genetic predisposition, muscle-aponeurotic dystrophy, collagen-metabolic disorder, smoking, obesity, age and concomitant diseases [1–5]. Hernias can be classified according to localization (groin, femoral, epigastric, umbilical, lumbal, as to left-sided, right-sided, unilateral, bilateral), possibility to reposition of hernias (reponible or unreponible), primary or recurrent, or direction of the hernias spread (indirect, direct or combined) [6]. Also, there are specific types of hernias (Littre, Richter, sliding, etc.) and lot of classifications in accordance to the principles of its authors [6–8]. A contemporary hernia's classification should be clear, simple, based on the hernia localization and diameter of the fascial defect. Also, the classification of hernias should contain preferable method of hernia solving (open surgery or minimally invasive surgery-laparoscopic) [7, 8].

The inguinal hernias are the most common hernias overall. They represent a protrusion of the content of abdominal cavity and/or pre-peritoneal fat through the hernia defect above inguinal ligament [4–7], whilst femoral hernia is a protrusion of the content of the abdominal cavity and/or preperitoneal fat below the inguinal ligament [5, 9]. Clinical symptoms vary significantly from asymptomatic hernia without pain or discomfort to the significant constraint and pain, and serious complications including incarceration and strangulation of hernia sack content [6, 10, 11].

The incidence and prevalence of groin hernias are unknown. However, the possibility that some person will undergo surgery of groin hernia during lifetime is very high with prevalence ranging from 1–30% [12]. Only treatment for groin hernias is surgical reparation through open or laparoscopic surgical approach. There are a lot of methods and their modifications of surgical reparation of groin hernias. Some of the most common open surgical used procedures are Lichtenstein and nylon-darn (Abrahamson) [1, 6]. Although they are not common, the postoperative and long term complications should be reduced to a minimum in order to provide less postoperative discomfort with short recovery, regardless of the type of surgical approach [13, 14].

This study was undertaken to evaluate the most prevalent surgical procedures and early postoperative complications after groin hernia reparation in large series of operated patients, and to assess the demographic characteristics of operated patients and morphologic characteristics of groin hernias.

Methods

This retrospective study included all operated patients with diagnosis of groin hernia in 4 years period (from 2009 to 2012) hospitalized at the Department of Surgery, Military Center in Novi Sad, Serbia. The analysis included 1,211 patients. In all patients a demographic characteristics including gender and age, clinical characteristics (symptoms and signs) and hernia type were analyzed. The patients were stratified in age groups with subsequent comparison between groups. All patients were operated by using an open surgical approach. The surgical procedures for hernia solving were analyzed and early postoperative complications were assessed. All statistical analysis was performed using SPSS software (Statistical package for the social sciences version 18.0, Chicago, IL, USA). χ^2 test was used to test the significance of differences between groups. Data are presented in numbers (percent). *P* values less than 0.05 were considered statistically significant for all comparisons.

Results

The study included 1,211 patients who underwent open surgical reparation of groin hernia. Elective surgery was performed in 1,207 (99.7 %) and urgent treatment was indicated in only 4 patients. In those patients, 3 were presented with inguinal incarcerated hernia and one with femoral incarcerated hernia. Total of the operated patients 1,127 (93.1%) were males and 84 (6.9%) were females with significant difference in gender ($\chi^2 = 898.306$; $p < 0.001$).

Groin hernia was found in 1,195 (98.7%) patients; there were 1,123 (94%) male and 72 (6%) female patients. Femoral hernia was found in 16 (1.3%) patients; there were 4 (25%) male and 12 (75%) female patients. See Table 1. The-

re was significant difference in distributions of inguinal and femoral hernia between genders ($\chi^2 = 116.342$; $p < 0.001$).

Bilateral hernia was present in 16 (1.3%) males, but there were no females with bilateral hernia. Primary hernia was present in 1,146 (94.6%) patients, whereas recurrent hernia was present in 65 (5.4%) patients. Patients were stratified in age groups (from 21 to above 81 years) and the structure of the patient's age is showed in Table 2.

There was no significant difference in patient's age and hernia occurrence. However, a significant positive trend of hernia occurrence and patients in the age group of 71 and above was found ($F = 24.905$ $p = 0.008$).

In male patients right sided inguinal hernia was present in 664 (57.6%) patients and left sided in 488 (42.4%) patients. In female patients right sided inguinal hernia was present in 7 patients and left sided in 5 patients. There were 16 patients with bilateral inguinal hernia, all males. There was no significant difference in side of inguinal hernia occurrence and gender ($\chi^2 = 0.182$; $p = 1.000$). The same percentage of male patients suffer from right sided and left sided femoral hernia. In females there were higher occurrence in femoral hernia on the right side then on the left side (7 : 5 patients), but without significant difference in hernia localization.

Surgical procedures for hernia reparation were: the Lichtenstein in 620 (51.2%) patients, the nylon-darn (Abrahamson) procedure in 358 (29.6%) patients, the Bassini in 196 (16.2%) patients, the Halsted in 17 (1.4%) patients, and the Lothaissen in 20 (1.7%) patients.

There were 12 patients with recurrent hernia of which 5 patients were males and 7 females. In those patients, 6 patients were primary operated applying the Lichtenstein procedure and other 6 applying the nylon-darn technique. Surgical reparation of recurrent groin hernia was the Lichtenstein procedure in 8 patients, the nylon-darn procedure in two patients and the Bassini procedure in two patients. Overall postoperative complications were presented in 78 (6.4%) of patients and they are shown in Table 3.

Wound infection was the most common postoperative complication recorded, and it occurred in 2.4% of patients. Among those patients, 16 were operated using the Lichtenstein procedure, 7 using the nylon-darn technique, 4 using the Bassini and 2 of them using the Halsted procedure without significant difference among patients in regard to operative techniques used ($p > 0.05$). Neuralgia as the second most common complication recorded, was observed in 10 patients operated using the Bassini technique, in 4 patients operated using nylon-darn technique and in 2 of them operated using the Lichtenstein procedure. Significant difference was found ($p = 0.018$) in neuralgia occurrence among patients operated using the Bassini procedure and other surgical techniques. Testicular atrophy was found in 2 patients, both of them were operated using the Lichtenstein technique. There was no perioperative mortality.

Table 1

Distribution of hernia type between genders

Gender distribution	Hernia		Total, n (%)
	inguinal	femoral	
Male, n (%)	1,123 (99.6)	4 (0.4)	1,127 (100.0)
Female, n (%)	72 (85.7)	12 (14.3)	84 (100.0)
Total, n (%)	1,195 (98.7)	16 (1.3)	1,211 (100.0)

Table 2

Structure of age in patients with groin hernia

Age (years)	Patients with groin hernia
	n (%)
21–30	87 (7.2)
31–40	78 (6.5)
41–50	143 (11.8)
51–60	273 (22.5)
61–70	330 (27.2)
71–80	259 (21.4)
81+	41 (3.4)
Total	1,211 (100.0)

Table 3

Postoperative complications in 1,211 operated patients with hernias

Complication	Patients, n (%)
Wound infection	29 (2.4)
Seroma	13 (1.07)
Hematoma	7 (0.58)
Urinary retention	5 (0.41)
Scrotal induration	6 (0.49)
Neuralgia	16 (1.32)
Testicular atrophy	2 (0.16)
Total	78 (6.4)

Discussion

Groin hernias are common pathology among men population, the most common pathology in children's population, with a new peak of incidence in early adult period (maximal physical activity), and the second peak in persons older than 65 years (weak structure of the connective tissue) [1, 3, 4, 12]. More than hundred years ago, the male/female ratio of groin hernia incidence was 20/1, and since that the incidence increases towards the female population. However, still the male/female ratio is not reduced much and amounts approximately 8–15/1 at the expense of the male population [12, 15]. Our results are similar showing that inguinal hernia repairs were carried out in total almost 14 times more commonly in the male than in the female population. As opposed to inguinal hernia, the femoral hernia in our study group was more common in the female population than in males (3 : 1) with significant difference in distribution of inguinal and femoral hernia between genders. These results correspond with the incidence of femoral hernia in general population over world [16]. The incidence of groin hernia is approximately 2% in men and 0.3% in women, whereas prevalence in men below 25 years is 18/100000, and increases at the age of 69–74 in up to 40/100,000, reaching 47/100,000 at the age above 75. For the entire population prevalence was reported as 24/100,000 [1, 9, 12, 17]. The age of patient has a strong influence on the incidence, etiopathogenesis and treatment inguinal hernia as well. In our study 71.1% of patients were in the age group from 51 to 80, and 27.2% of patients were in the age group of 61–70, suggesting weak structure of connective tissue in older population as etiology factor of hernia occurrence, as reported recently [12].

More than hundred years after revolutionary rebound in groin hernia surgery initiated by Bassini's operation, surgeons developed many variations of different techniques, tensional or non-tensional, open or laparoscopic. During past two decades, only non-tensional techniques have been applied, but with variable recurrence rates ranging from under 1% to up to 10% [18, 19]. The usage of prosthetic material in hernia surgery led to changes fundamental in the surgical strategy, because the concept of covering miopectineal orifice with non-absorbable prosthesis brought minimal incidence of recurrence [20]. Principles of surgical treatment should be based on right indication and proper selection of a surgical technique, in every individual case.

The most commonly used open surgical techniques in reparation of groin hernia in the last several decades includes the Lichtenstein procedure and plication-darn (nylon-darn) technique. To decrease the tension in the suture line, Lichtenstein added a synthetic mesh and sutured from the edges. Hernia repair in this manner have made the initial recurrence rate drop to less than 1% [21]. However, the recurrence rates have been raised in further series up to 8% [22, 23]. The explanation for raising recurrence rates lied in complications of the synthetic mesh which include fibrosis and chronic inguinal pain, chronic infection and shrinking of the mesh [24]. In 1946, Maloney et al. [25], originally developed a new method of herniorrhaphy, namely the Darn repair, by continuous suture with monofilament nylon. It was a tension-free suture adapted from the Bassini technique. This technique popularized by Abrahamson in the seventies with very low recurrence rate [26].

The longest study about this method was a 23-year study reported by El-Bakry [27] who found the recurrence rate of 0.2% in 600 patients. The main disadvantage in our study was short term follow-up without assessment of recurrence rate of operated patients. However, a large number of operated patients allowed us to make the comparison among surgical techniques for groin hernia reparation. Since the first series of laparoscopic hernia repairs were published in 1990, this minimally invasive surgical approach for groin hernia solving has widely been used. According to the present guidelines of the European Hernia Society the Lichtenstein or endoscopic repair should be the procedure of choice for both primary unilateral and bilateral inguinal hernias with the note that endoscopic repair should only be performed if expertise is available [10].

Decision on the type of a surgical technique for groin hernia reparation in our series was based on the hernia size and type, thickness of the musculoaponeurotic layer, surgical skills and experience. The majority of patients in our study were operated using the Lichtenstein procedure, 51.2% of all operated patients, and the second most common surgical technique was the nylon-darn method in 358 patients. Although in literature there are data of higher complication rates after the Lichtenstein hernioplasty [24], it is certain that careful and meticulous technique could reduce a number of postoperative complications whether the Lichtenstein or darn procedure was used. However, for a good hernia repair, either mesh has to be sutured without inducing foreign body reaction or other type of reparation without tension has to be performed. The nylon-darn method for groin hernia reparation fulfils these conditions.

The most common early postoperative complication in our study was wound infection. It was reported that wound infection or superficial infection is more common in the Lichtenstein than in the darn technique, but without significant difference [28]. Although we noted more patients with wound infection operated using the Lichtenstein than those using the nylon-darn technique, there were almost twice more patients operated by using the Lichtenstein procedure. Actually, 16 patients in the Lichtenstein group and 7 patients in the nylon-darn group had wound infection, which was 2.5% and 1.95% in incidence rate of wound infection in each group, without significant difference between the groups. Significant higher occurrence of neuralgia was found in patients operated using the Bassini procedure, which suggests more common nerve entrapment in this method.

Conclusion

Groin hernias are frequent pathology in general population. Prevalence of groin hernias is higher in men population, while femoral hernias are more common among women. The population which is the most affected is in the age of 61 to 80 years. The most commonly used open surgical procedures

for groin hernia reparation are the Lichtenstein and the nylon-darn. Both methods have low and similar incidence rates of postoperative complications. Adequate technique for groin hernia reparation should be selected on the basis of anatomical findings during surgery and experience and skills of the surgeon.

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A new pathophysiological concept and new classification of pre-eclampsia

Novi koncept patofiziologije i nova klasifikacija preeklampsije

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Key words:
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Ključne reči:
preeklampsija; dijagnoza; klasifikacija.

Introduction

The first description of eclampsia (E) was given by Hippocrates, a father of modern medicine (460-377 BC), a son of Heraclides from the island of Kos [1]. After more than two millennia since the first descriptions, the syndrome of pre-eclampsia/eclampsia (PE/E) has remained a multi-system disorder of unknown etiology. The diagnosis is based on a clinical picture and laboratory analysis; an efficient prevention and screening are missing, the therapy is symptomatic, while giving birth still remains the only causal therapy.

Hypertensive disease in pregnancy (HDP) implies various clinical entities with hypertension being the common one. Thirty-one epidemiological studies have been published in the period from 1979 to 2013 with the incidence of PE on the global level in five different regions of the World Health Organisation and in 29 countries amounting to 2.16%, while the incidence of E amounts to 0.28% [2]. In Europe, more than 90% of deaths of mothers caused by PE/E could have been avoided [3, 4]. Pregnant women having PE/E have a greater incidence of induced births, C-sections and preterm births [2]. In women with E, an exponential risk growth for death or high threat to the life of the pregnant woman, fetal death, neonatal death, perinatal death and reception to the neonatal intensive care unit has been detected [2].

Why is the problem still significant? The PE incidence has grown by 25% in some of the developed Western world countries [5]. Another major reason is the estimate that every year 50,000–60,000 women in the world die from PE and its complications [6, 7]. For each of these deaths one must add 50–100 pregnant women whose life is threatened due to PE/E [8, 9]. PE represents a major reason of iatrogenic prematurity while the last but not the least important reason is that PE has been recognised as a serious risk factor for the appearance of cardiovascular and metabolic diseases in the later life of the woman and her new-born [10, 11].

The etiology and pathophysiology of pre-eclampsia

How can we define in the simpliest way the pathophysiological PE mechanism today? Pre-eclampsia is a disease of the placenta from which both the mother and a fetus suffer. This definition fulfils the criterion of simplicity, but unfortunately it is not scientifically sufficient, i.e. why, how, when? Much is known today and if we were to explain PE in the shortest possible way, nowadays we can summarise: various genetic and epigenetic factors have an influence on an inappropriate spiral artery remodelling process, i.e. bad placentation, which as a consequence has a bad placenta perfusion and the appearance of oxidation placenta stress, which stimulates the synthesis of different humoral mediators leading to endothelic dysfunction of different organs and organic systems of the pregnant woman and the fetus, presenting itself as a multi-system disease, which PE today definitely is (Figure 1).

The basic problem in studying the aetiology and pathophysiology of pre-eclampsia is the non-existence of the uniform HDP criteria even though in the past decades there have been several attempts to introduce the unique criteria by different international associations studying HDP. Another aggravating circumstance is the issue whether PE is one or several diseases. Just like HELLP (haemolysis, low thrombo-

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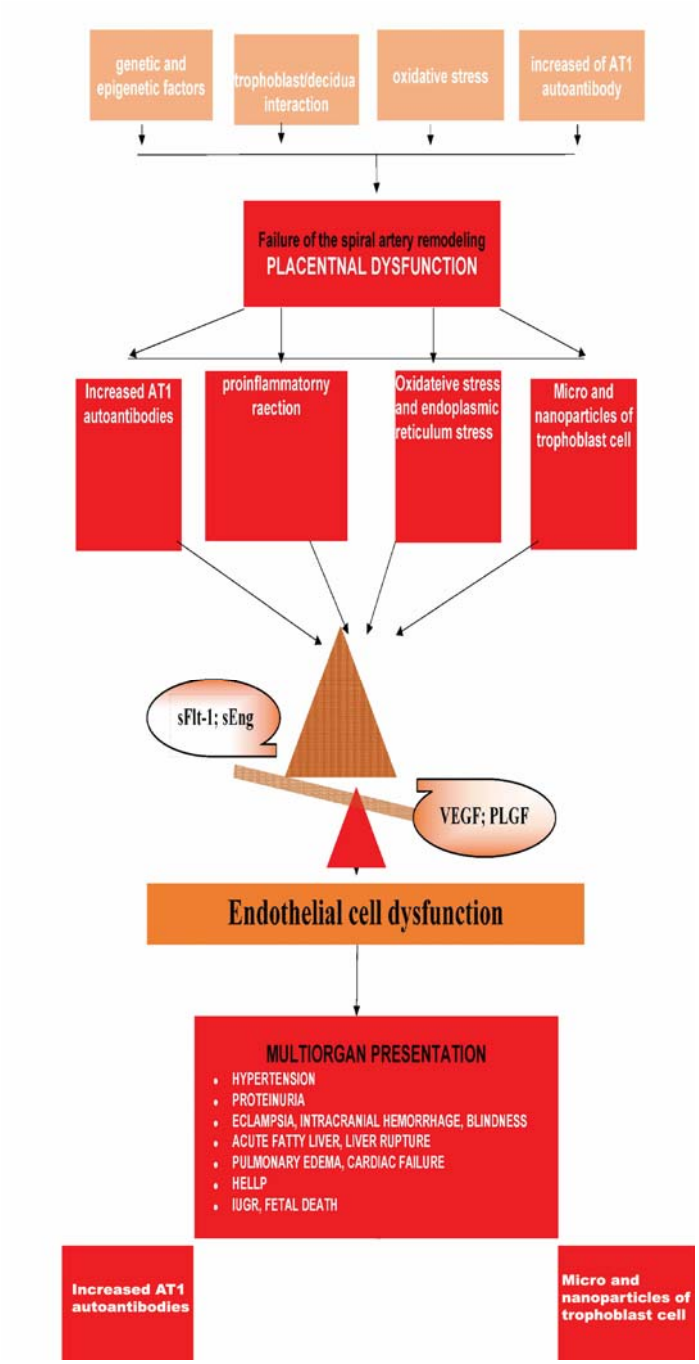

Fig. 1 – The pathophysiology of preeclampsia.
sFlt-1 – the soluble fms-like tyrosine kinase 1; sEng – the soluble endoglin; PLGF – the placental growth factor; VEGF – the vascular endothelial growth factor; AT1 – the angiotensin receptors 1; IUGR – the intrauterine growth restriction; HELLP-hemolysis, elevated liver enzymes, low platelet count.

cytes and increased liver enzymes) separated itself as a special entity, the early and late PE can already be considered separate entities. Basically, the new classification primarily leans on the new understanding of PE pathophysiology and extensive epidemiological studies.

Spiral artery remodeling

The human placenta is a temporary organ, one of the most vascular organs, which is made of a tissue that is 98% of fetal, i.e. trophoblast origin; only approximately 2% are of decidua, uterine, mother's origin. The length of the capillary system of the placenta at the end of the pregnancy amounts to approximately 550 km and its surface is approximately 12 m^2 [12]. The capillary surface of the placenta is essential for the growth and development of the fetus. The vasculogenesis starts 3 weeks after conception, so as for the fetoplacental circulation to be established, around 8 weeks of gestation [13].

An appropriate development of trophoblasts on one side and the adjustment of blood vessels of the uterus on the

other, are conditions for a normal human pregnancy development. The trophoblast is a tissue originating from a fertilised egg cell, carrying the genetic embryo constellation. There are three types of trophoblasts: 1) syncytiotrophoblast (STB), 2) cytotrophoblast (CTB), 3) extravilloustrophoblast (EVT). EVT proliferates from the so-called chorionic villi in charge for the stabilisation and fixation of the placenta, as opposed to the freely floating chorionic villi submerged in intervillous space providing spiral arteries with blood. EVT has the features of the invasive tissue which will spread into the uterus stroma. The goal of the EVT invasion is that in the uterus stroma it reaches the spiral arteries.

The first description of spiral arteries was given by the Hunter brothers, William (1718–1783) and John (1728–1793) Hunter, in their masterpiece "Anatomy of the Human Gravid Uterus" published far back in 1774 [14]. A century and a half ago, it was speculated that the spiral arteries undergo a change in their structure during pregnancy; in 1927 Otto Grosser [15] for the first time came up with the idea that these new cells, remodelling the spiral artery wall, are aretrophoblast cells. Nevertheless, it was scientifically proven that the "new cells" in the spiral artery walls are trophoblasts only with the introduction of cytokeratin immunohistochemical tests, which have finally confirmed the trophoblast origin of endovascular and intramural cells in the spiral artery wall. The process of trophoblast invasion, to which the spiral arteries are subjected, implies a loss of endothelial cells of the spiral arteries, loss of elastic lamina as well as a loss in the muscular layer, which is replaced by fibrinoid layers [16]. The wall of the changed arteries becomes thinner, softer and has a large capacity for passive dilatation, while the lumen of spiral arteries becomes expanded after trophoblast invasion so that the blood stream is larger. At the same time, the remodelled endothelium of spiral arteries becomes insensitive to the vasoconstrictors. The remodelling takes place in decidua but also in the myometrial segment, on average in approximately 100 spiral arteries of a placenta.

The sense of adequate remodelling of spiral arteries is to transform the placenta into a large capacity and low pressure organ and for the spiral arteries primarily to become insensitive to regulatory mechanisms of mother's blood pressure. The spiral artery remodelling process takes place on two occasions (the "two wave invasion" theory). The first wave of the EVT invasion (from the so-called anchoring chorionic villi) takes place only in the deciduas of the spiral arteries from 8–10 weeks of gestation. The second wave of EVT invasion happens between 16 and 18 weeks of gestation. The EVT invasion in this second wave takes place in the deeper myometrial spiral artery segment [17].

The EVT invasion process into the spiral artery wall, beside two invasion waves, takes place also from two directions: 1) interstitial and 2) endovascular. Thus, it can be said that the spiral artery wall is exposed to the trophoblast EVT invasion both from "outside" and from the "inside", i.e. interstitially and endovascularly.

It remains an open question as to why the inadequate spiral artery remodelling process through trophoblast invasion in certain cases leads to the manifestation of the clinical picture of PE, while sometimes it is an intrauterine fetal growth restriction (IUGR), and sometimes an early birth (PTP) [18, 19]. It can nowadays be said that the most important obstetric entities: PE, PTP, placental abruption, preterm premature rupture of fetal membranes (PPROM) and late miscarriages are results of "deep placentation disorders", i.e. inadequate remodelling of spiral arteries in the deep myometrial segment [20]. It is obvious that the spiral artery remodelling process is not a process taking place according to the all or nothing principle [16].

Oxidative stress

Placental insufficiency results in oxidative stress (OS). Pre-eclampsia is characterised by an excessive production of free radicals and/or non-existence of a satisfactory antioxidative capacity [21–24]. Oxidative stress can be simply defined as a misbalance in the production of oxidants (free radicals and reactive metabolites) and their elimination, i.e. the protective mechanism of the antioxidative system. The oxidants include the reactive forms of oxygen (ROS) and the reactive forms of nitrogen (RNS). ROS and RNS promptly react with lipids, proteins and DNA cell molecules, thus manifesting harmful effects. The mitochondria are one of the most important sources of ROS in trophoblast cells but they are also the most important place of their action. It is conventional wisdom that the oxidative stress is always harmful. Oxidative stress plays a very complex and significant role in the signal modulation processes, emphasises the synthesis of antioxidant enzymes and impacts the reparation processes, inflammation, apoptosis and cell proliferation [25]. In the first trimester, the embryo develops in a low oxygen environment as opposed to the second trimester when there is a significantly larger exchange in oxygen on the level of the placenta in order to meet the needs of the growing fetus. It is precisely this low oxygenation that is significant for the proliferation of trophoblasts in the first trimester. The experiments of Genbačev et al. [26] have shown that the low oxygenation of trophoblasts in the first trimester has an impact on the good proliferation of trophoblasts but not on its invasivity and differentiation [26].

OS have and impact on autophagy and apoptosis, the two key interrelated processes. Autophagy is a protective while apoptosis is a destructive process at the level of the placenta. Autophagy has recently become one of the most interesting and most studied processes [27]. It is considered that autophagy is a self-regulating, catabolic process with the aim to remove the undesirable proteins, damaged organelles and their harmful products. According to the most recent understanding, autophagy represents the most important protection of trophoblast cells in the conditions of OS. Contrary to the conditions of OS, apoptosis is activated in a complex way which has as a consequence a programmed cell death of trophoblast cells and inadequate remodelling of spiral arteries. Lately, the importance of the endoplasmic reticulum (ER) stress which occurs as a result of ischemia of intervillous space due to inadequate remodelling of spiral arteries is emphasised. The ER stress, as a consequence, has an inadequate

posttranslational protein modification and their insufficient "folding" [28]. There is a problem of the so-called unfolded protein response or UPR) [29]. UPR further leads to the end of trophoblast cell proliferation and if UPR is present, it leads to apoptosis. The trophoblast apoptosis has as a consequence the release of micro and nanoparticles in the maternal circulation, which possess the ability of stimulating a proinflammatory response [30]. These two processes are mutually connected in a complex way and their balance plays a major role in the placental homeostasis [31].

Oxidative stress triggers also other humoral processes: proinflammatory response and release of cytokines: tumour necrotising factor (TNF)-alpha, interleukins (IL-6), (IL-2) [32–34]; activation of complements [35]; stimulation of the synthesis of antiangiogenic factors: soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), reducing the production of placental growth factor (PLGF) [36–38].

Since the intravascular inflammation, beside in PE, can also be found in other obstetric syndromes such as preterm birth [39], PPROM [40] IUGR [41, 42] and pyelonephritis [43], without hypertension and proteinuria, so that the conclusion imposes itself that the very inflammation exists in pre-eclampsia, but that it is not sufficient to cause the disease symptoms. Nowadays, the prevalent opinion is that the placental hypoxia leads to the release of antiangiogenic factors sFlt-1 and sEngwhich together with the proinflammatory cytokines lead to endothelial activation and vasospasm, i.e. to endothelial dysfunction [44]. It has been shown lately that the increased maternal systemic proinflammatory response in PE does not correlate with the level of antiangiogenic sFlt-1 and sEng [45]. It is not known what the interaction between the inflammatory and the angiogenic system is, however, the possibility that the inflammatory system stimulates the angiogenic system and vice versa is not excluded.

Angiogenic factors

Since Maynard and associates in 2003 published that excessive placenta production of sFlt-1 represents a major factor in the PE pathophysiology, the literature is considering different major functions of these important biomarkers. sFlt-1 is a soluble formof receptor belonging to the vascular endothelial growth factor (VEGF group) of the receptor (VEGFR-1). sFlt-1 is exprimated in an insoluble form (Flt-1) on endothelial cell membranesand placenta (mostly on syncytiotrophoblast cells) (Figure 2). VEGF has an important function in the development of endothelium, its proliferation, vascular permeability and fenestration of endothelial cells. VEGF realises its function by connecting to insoluble Flt-1receptors on the endothelium. If the soluble form of Flt-1 receptors is in increased concentration, VEGF will tie to them and in this way its connection on the receptors on the endothelium will fail. In this way, also the positive impact of VEGF on the endothelial cells will fail, too. Placental growth factor (PLGF) is also a member of the VEGF family with a strong proangiogenic, positive action on endothelial cells,

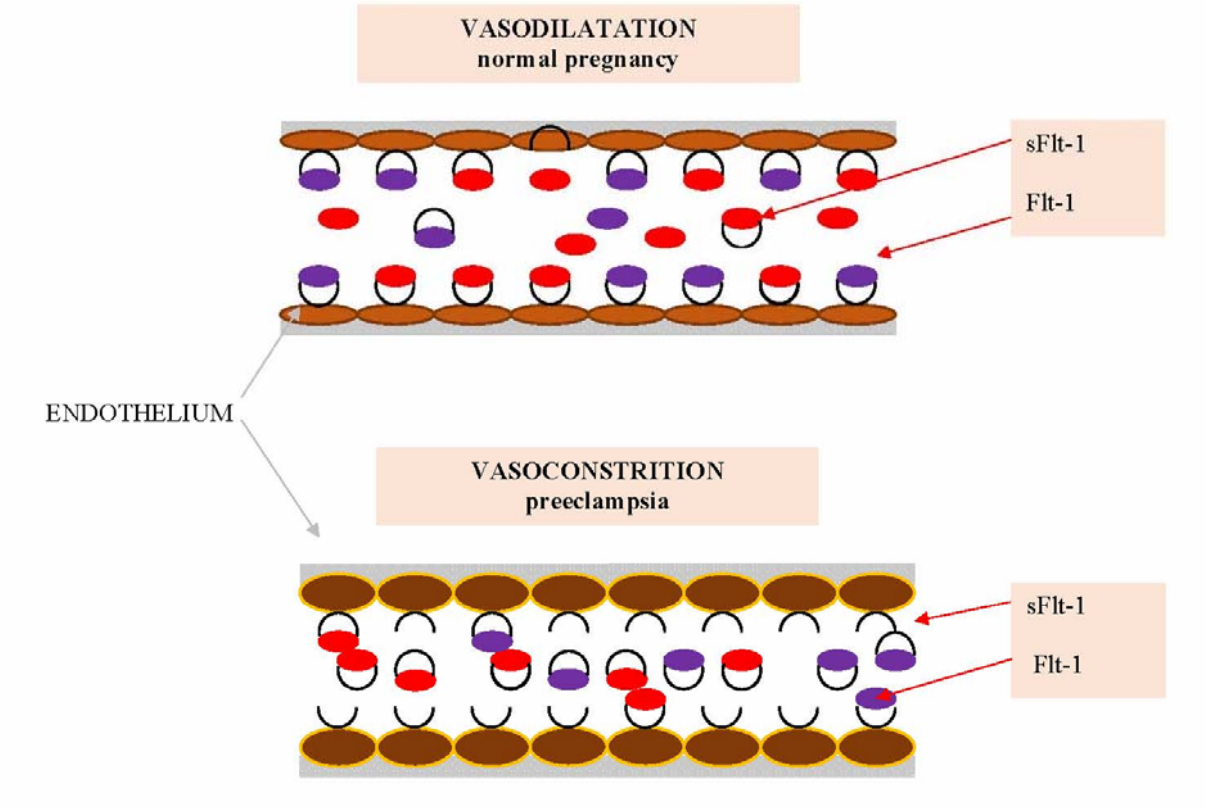

Fig. 2 – The functioning of the placental growth factor and vascular endothelial growth factor. PLGF – placental growth factor; VEGF – vascular endothelial growth factor; sFlt-1 – soluble fms-like tyrosine kinase 1; Flt-1 – non soluble fms-like tyrosine kinase 1.

which realise its function also through the same receptors as VEGF but in a somewhat modified way. [46] In the same way sFlt-1 is connected to the PLGF molecules, reduces its concentration and, thus, its positive effect on endothelial cells is missed.

Primarily, light is shed on the major role of angiogenic factors in explaining the PE pathophysiology. In the past five years in the literature there has been a significant number of papers discussing the role of angiogenic factors in predicting PE, differential diagnosis and HDP classification; recently, the possible clinical use of the sFlt-1/PLGF ratio in the prediction of unfavourable perinatal PE complicated pregnancy outcome is of major importance.

In PE, the concentration of antiangiogenic factors is growing (sFlt-1 and sEng), while the concentration of proangiogenic factors PLGF and VEGF is decreasing. PLGF and VEGF have a positive effect on the endothelium in pregnancy but in different time periods and in different ways [47]. VEGF has a major role in the branching of angiogenesis, it stimulates endothelial proliferation and migration in the first trimester of pregnancy, while PLGF helps the so-called angiogenesis without the branching of angiogenesis (non-branching angiogenesis) in the second and third pregnancy trimester [47]. Since in the first trimester there is the condition of lesser oxygenation, each hyperfusion and hyperoxigenation may block the VEGF level and lead to early PLGF pick which has as a consequence inadequate blood vessel branching of trophoblast villi and may lead to the pregnancy's development being stopped (Figure 3) [47, 48].

sFlt-1/PLGF ratio has better diagnostic performances than the analysis of individual sFlt-1 or PLGF markers [49–52]. simply and quickly define these markers and have been in commercial use since 2010 [49]. These markers have been incorporated into the German PE guide [58] and formally there are no official recommendations, so that this test has not yet entered the official clinical protocols.

Roberts et al. [59] stated, back in 1989, long before realising the role of angiogenic factors, the theory on PE as a disease of endothelial cells [59]. Back then, there was no sufficient explanation on which "toxin" in the mother's blood leads to the disease of the endothelium. The concept of endothelial dysfunction is a valid dogma even today.

Out of the large number of biomarkers, PE, sFlt-1, and sEng represent the most important biomarkers leading to endothelial dysfunction. Experimental studies on animals have shown that the elevated level of the circulating sFlt-1 may lead to presentation of all the characteristics of human PE: hypertension, proteinuria, brain oedema, haematological disorders and fetal development restriction [60–63]. Thadhani et al. [64] associates have shown that by removing sFlt-1 through apheresis from the pregnant woman's plasma, one mitigates the clinical manifestations of PE.

Nowadays, it is considered that the PE mechanism is a complicated and complex one; therefore, one has suggested a hypothesis of a combined excessive inflammatory response and disbalance of angiogenic factors. It is assumed that the inflammatory mediators act locally through autocrine or paracrine mechanisms leading to the apliphication effect of angiogenic factors [65].

Nevertheless, the antiangiogenic status of the mother does not always have PE as a consequence. The reasons for such a response are not clear, however, it is assumed that it is necessary to cross the individual threshold of disbalance of pro- and antiangiogenic factors. It is necessary to realise a sufficiently large production of antiangiogenic factors; their prolonged action is necessary but also the constitutional sensitivity of the maternal endothelium on the action of antiangiogenic factors. There is no

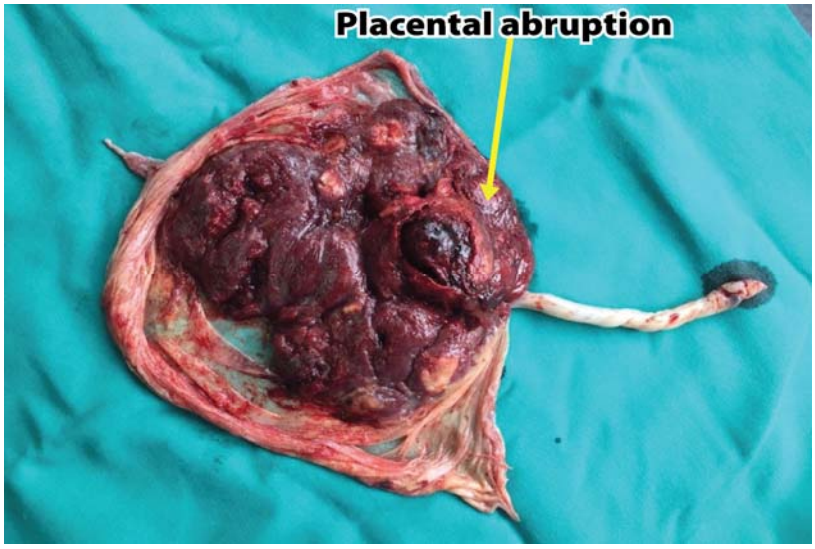

Fig. 3 – Preeclamptic placenta

This relationship represents one of the most important, new, laboratory tests pointing to the need of an urgent birth in pregnant women with PE and predicts an unfavourable outcome of the pregnancy complicated through PE [50, 53–57]. The application of sFlt-1/PLGF relationship in practice has been made possible by the introduction of automatic tests which

response to all questions but further potential clinical use of analysing (PLGF, sFlt-1, sEng) is yet to be expected [66].

Antibodies on angiotensin II receptors (AT)

As opposed to a normal pregnancy which is characterised by reduced sensitivity of endothelium on angiotensin II, in pregnant women with PE, due to genetic factors, immunomodulation, and external factors, there is an excessive sensitivity on angiotensin II [67, 68]. This sensitivity can be detected even before 24 weeks of gestation. It was established that some pregnant women with PE create autoantibodies on type one angiotensin II receptors (AT1). Antibodies on AT1 receptors injected to pregnant rats lead to hypertension, proteinuria, and increased levels in sFlt-1 and sEng [69]. Antibodies on AT1 receptors ead to the occurrence of hypertension through the activation of complements and through the stimulation of antiangiogenic factor production, sFlt-1 and sEng [70]. Lack of immunoassay for these specific AT1 receptors disturbs further understanding of their role and their possible application in clinical practice.

Activation of trombocytes and thrombin

Thrombocytopenia is one of the prognostically most unfavourable laboratory indicators in PE [71]. Thrombocytopenia sometimes precedes the occurrence of clinical signs of PE [71]. It is considered that a reduced number of thrombocytes occur as a result of different factors: increase in the size of the thrombocytes, shorter life of the thrombocytes, increase in the thrombocyte factor 4 or due to increased production of thrombocyte thromboxane B2 [72]. Vasoconstriction and thrombocytopenia in PE most probably occur as a result of reducing the prostacyclin synthesis [73]. The increase in vasoconstriction thromboxane A2 and reduction of vasodilation prostacyclin can be found in PE [74]. The activation of thrombocytes can lead to the creation of thrombi in the microcirculation of different organs and placenta.

One of the major characteristics of PE is the activation of coagulation cascade [75]. In literature there is a multitude of explanations for excessive creation of thrombin in PE [76]. The following is listed as a reason: endothelial dysfunction, activation of thrombocytes, monocyte chemotaxis, lymphocyte proliferation and neutrophil activation but also an increased synthesis of tissue coagulation factors that are released under the influence of proinflammatory cytokines. The thrombin leads to a creation of fibrin deposits in different organs in PE. Excessive creation of thrombin may range from subclinical to the occurrence of disseminated intravascular coagulation as one of the most serious PE complications. The excessive creation of thrombin can be monitored in laboratory by defining the concentration of thrombin-antithrombin (TAT) complex or by defining antithrombin III [76, 77].

Pre-eclampsia genetics

The hereditary factor has been for a long time recognised as the starting important event in the occurrence of PE. It is still unknown in which way the inheritance takes place and which genes are responsible. Molecular research has the capacity to provide indications of the basic causes of PE which are not available through other research methods. Such a strategy has been made possible by the expansive development and merging of molecular biology and information technologies. Two approaches are used for the purpose: testing the gene polymorphism in the candidate, while the other approach implies integrational systemic study of the entire human genome. The literature presents a large number of polymorphisms of different candidates' genes.

MicroRNAs (MiRNAs) are non-coding RNA segments of a size of 21–25 nucleotide bases, for which it is considered that they post-translationally regulated the gene expression [78]. MiRNAs are included in the regulation of trophoblast proliferation, apoptosis, migration and invasion [79]. MiRNAs are significant also in the regulation of angiogenesis [80, 81].

The plasma concentration of free DNA (cfDNA) fragments, as well as free fragments of fetal DNA (cffDNA) have their place in screening, detection, but also in the prediction of an unfavourable perinatal PE outcome [82].

Classification of hypertension diseases in pregnancy

The HDP classification has a major importance for the study of all of the aspects of hypertension disease in pregnancy. In the literature there is a large number of different HDP classifications, both on the part of national and on the part of various international associations [83–88]. The confusion significantly slows down the basic research in PE etiology and pathophysiology and also impacts the recommendations and prevention protocols as well as the treatment of the hypertension disease in pregnancy. Since 2013, after a guide was published by the American College of Obstetricians and Gynaecologists (ACOG) workgroup, there has been a tendency of wider implementation of new diagnostic criteria into national guides also of other countries and associations [89]. This classification basically and primarily leans on the new understanding of PE pathophysiology and also to extensive epidemiological studies. The ACOG HDP classification has the tendency to be simple, precise and easy for clinical application. According to this classification, HDP can be divided into four basic groups: 1) Pre-eclampsia/eclampsia; 2) Chronic hypertension; 3) Chronic hypertension with superimposed pre-eclampsia; 4) Gestational hypertension.

Pre-eclampsia/eclampsia diagnosis

Pre-eclampsiais is a specific form of hypertension in pregnancy having a multi-system presentation. It can be said that the PE syndrome, which is primarily characterised by the appearance of hypertension after 20 gestation weeks in previously normotensive women that mostly comes together with proteinuria but may also be associated with most varied other symptoms and signs.

Hypertension and proteinuria have earlier been two basic, classic criteria for making the PE diagnosis. Beside the

above two classic PE criteria, some expecting mothers, beside hypertension, also have multi-system symptoms or signs which even without the presence of proteinuria point to a severe form of PE [89].

Pre-eclampsia manifests itself as early and late PE; these forms are nowadays considered as various PE entities/subgroups [90]. Early PE manifests itself before 34 gestation weeks, while late PE manifests itself after 34 gestation weeks [4, 91, 92]. The maternal and fetal morbidity and mortality are far more frequent in the subgroup of early PE, before 34 gestation weeks [4, 91]. The basic differences between early and late PE are presented in the Table 1.

The most important in the new classification of PE is that proteinuria is no longer the basic and necessary criterion for diagnosing PE with elevated blood pressure ≥140/90 mmHg [93, 94]. Proteinuria ≥ 5 g and IUGR are no longer the criteria for diagnosing severe PE. Another important fact is the introduction of the indicators of multi-system endothelial dysfunction as equal criteria for diagnosing PE.

According to the new ACOG classification, PE is defined as elevated systolic blood pressure of 140 mm Hg or diastolic blood pressure of 90 mm Hg or more, or both the systolic and the diastolic blood pressure are above ≥140/90 mm Hg, measured twice with a gap of 4 h (when one measures the blood pressure value ≥ 160/110 mm Hg, the next measurement can follow immediately, in just a few minutes, for the purpose of introducing antihypertensive therapy) with proteinuria in 24 h-urine ≥ 300 mg or protein/creatinine ratio ≥ 0.3 or in absence of quantitative methods one can use the read proteins in urine on a test tape 1+. In absence of proteinuria, in order to diagnose PE it is necessary that, beside hypertension, there is at least one of the following criteria: thrombocytopenia (number of thrombocytesis less than 100.000/μL), kidney insufficiency (concentration of creatinine in serum above 97 μmol/L), decreased liver function (enzyme activity AST and ALT twice higher than the upper limit of the referential interval), appearance of lung oedema- or appearance of cerebral, i.e. visual symptoms (Table 2) [89].

Diagnosing severe pre-eclampsia

Several different approaches have earlier been used by different associations in order to evaluate the severity of pre-eclampsia. According to the English The National Institute for Health and Clinical Excellence (NICE) [95] the severity of PE is evaluated only by basing it on blood pressure, while ACOG in 2002 estimated the severity of PE by basing it on blood pressure, but also on other indicators as presented in the Table 3 [85].

According to the new ACOG classification of 2013, the use of the term moderate PE is not recommended but rather "PE without severe PE characteristics", while severe PE is one in which, according to ACOG, beside the basic criterion of elevated systolic blood pressure 140–160 mm Hg and/or diastolic blood pressure 90–110 mmHg also fulfils one of the following criteria: thrombocytopenia (a number of thrombocytes is lower than 100,000/μL), kidney insufficiency (concentration of creatinine in the serum above 97 μmol/L), liver insufficiency (enzyme activity AST and ALT twice higher than the upper limit of the referential interval), appearance of the lung oedema or appearance of cerebral, i.e. visual symptoms. Severe pre-eclampsia is also elevated blood pressure ≥ 160/110 combined with proteinuria or some of the other criteria of severe PE as presented in the Table 4.

Severe PE increases the morbidity and mortality of the mother and fetus and the characteristics of severe PE stated in theTable 4, when developed in the clinical picture, represent prognostically unfavourable, major factors, which make severe PE a serious disease, with a larger risk for an unfavourable outcome [96]. One cannot but notice that proteinuria ≥ 5g /24, as was already mentioned, is no longer a criterion for diagnosing severe PE. Moreover, IUGR is no longer conside

Table 1

Basic differences between early and late preeclampsia

Early onset preeclampsia (≤ 34 weeks of gestation)	Late onset preeclampsia (≥ 34 weeks of gestation)
A fetal diseases that is typically associated with placental dysfunction	Maternal disorder due to underlying maternal constitutional factors
Reduction in placental volume (Figure 3)	Normal placental volume
IUGR	Normal fetal growth
Abnormal uterine and umbilical artery Doppler measurement	Normal uterine and umbilical artery Doppler evaluation
Adverse maternal and fetal outcomes	Favorable maternal and neonatal outcomes
Low birth weight	Normal birth weight

IUGR – intrauterine growth restriction.

Table 2

American College of Obstehricians and Gynecologists (ACOG 2013) diagnostic criteria for preeclampsia

Blood pressure	- systolic ≥140 mmHg or diastolic ≥ 90 mmHg on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure - systolic ≥ 160 mmHg or diastolic ≥ 110 mmHg, confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy
Proteinuria	- ≥ 300 mg per 24 h or - P/K ratio ≥ 0.3 - dipstick 1+ (used only if other quantitative methods not available)
Or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following	
Thrombocytopeni	≤ 100 x 10^6/L
Renal insufficiency	serum creatinine > 97μmol/L, or a doubling of serum creatinine concentration in the absence of the renal disease
Impaired liver function	elevated blood concentrations of liver transaminases to twice normal concentration
Pulmonary edema	
Cerebral or visual symptoms	

Table 3

Severity classification of preeclampsia by the National Institute for Hcalth and Clinical Exellence (NICE 2010) and the American College of Obstetricians and Gynecologists (ACOG 2002)

NICE (2010)	ACOG (2002)
Mild systolic 140–149 mmHg and/or diastolic 90–99 mmHg	Mild to mederate systolic 140–159 mmHg and/or diastolic 90–109 mmHg
Moderate systolic 150–159 mmHg and/or diastolic 100–109 mmHg	Severe (any two if present)
Severe systolic ≥ 160 mmHg and/or diastolic ≥ 110mmHg	systolic ≥ 160 mmHg and/or diastolic ≥ 110 mmHg proteinuria ≥ 5gr/24 h or 3 +++ oliguria ≤ 500 mL/24 h cerebral or visual symptoms pulmonary edema or cyanosis epigastric or upper quadrant pain impaired liver function thrombocytopenia

NICE – The National Institute for Health and Clinical Excellence; ACOG –The American College of Obstetricians and Gynecologists.

Table 4

The American College of Obstetricians and Gynecologists (ACOG 2013) Severe characteristics of preeclampsia

	Severe characteristics of preeclampsia (any of these characteristics)
Blood pressure	systolic ≥ 160 mmHg or diastolic ≥ 110 mmHg on two occasions at least 4 h apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time)
Thrombocytopenia	≤ 100 x 10^6/L
Progresisve renal insufficiency	serum creatinine > 97μmol/L, or a doubling of serum creatinine concentration in the absence of the renal disease
Liver insufficiency	elevated blood concentrations of liver transaminases to twice normal concentration or severe epigastric or upper quadrant pain
Pulmonary edema	
New-onset cerebral or visual disturbances	

ACOG – The American College of Obstetricians and Gynecologists; AST – aspartate aminotransferase; ALT – alanine aminotransferase.

red a criterion for severe PE because for IUGR there are special clinical guides.

Since it has become clear that PE is a multi-system disease due to endothelial dysfunction, it no longer surprises that there is a series of the so-called atypical forms of pre-eclampsia. This group of atypical PE includes pre-eclampsia without proteinuria, normotensive PE, PE before 20 gestation weeks but also PE manifested postpartum [97].

Gestational hypertension has been marked with elevated blood pressure ≥ 140/90 mm Hg, without proteinuria, in pregnant women after 20 weeks of gestation, which used to be normotensive.

Eclampsia is the occurrence of tonic-clonic convulsive seizures in pregnant women with PE during pregnancy, during birth or immediately after birth.

Hypertension before conception or hypertension diagnosed in the first half of the pregnancy, before 20 ng ≥ 140/90 mm Hg, is classified as chronic hypertension [98].

Superimposed pre-eclampsia diagnosis

In 17–25% pregnant women with chronic hypertension, a superimposed PE will also develop. In 50% of these pregnant women, PE will develop before 34 weeks of gestation [98]. Pregnant women with superimposed PE have a worse forecast than those having only PE or only chronic hypertension. Establishing the diagnosis of superimposed PE is very often debatable and wrong [99]. According to the ACOG work group, the diagnosis of superimposed PE is certainly possible in the following situations: sudden worsening of hypertension or the need to increase the therapy, while the regulation used to be good with smaller dosage of medicines; increased liver enzymes; decrease in the number of thrombocytes ≤ 100×10^6/L; pain in the upper right quadrant or occurrence of severe headache; pulmonic congestion or lung oedema; renal insufficiency measured by the increase of serum creatinine ≥ 97μmol/L; sudden occurrence of proteinuria or its major aggravation.

If the blood pressure is only elevated, ≤ 160/110 mm Hg and if there is proteinuria, the superimposed PE can be marked as superimposed PE without the characteristics of severe PE. It is recommended to take care about the patient according to the PE protocol without any characteristics of severe pre-eclampsia. However, if beside chronic hypertension, there are manifestations of systemic damage, i.e. symptoms of severe PE (Table 4), the superimposed PE should be marked as the superimposed PE with characteristics of severe PE and should be taken care of according to the protocol for severe PE. From the classification aspect, both these forms are marked as superimposed PE; however, they will not be treated in the same way, as already stated [89].

Conclusion

Pre-eclampsia remains one of the most important obstetric entities. In the last decade, there has been a major progress in shedding light on the pathophysiology of pre-eclampsia. As a result of this knowledge, there has been a new approach to the classification of the hypertensive disease in pregnancy. Wider application of the recommended criteria by the American College of Obstetricians and Gynaecologists may assist in further studying of the aetiology and pathophysiology of pre-eclampsia but it must primarily enable the introduction of unique therapy protocols, as well as the prediction and prevention of pre-eclampsia.

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CASE REPORTS

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Primary intestinal lymphangiectasia in adults – diagnostic and therapeutic challenge

Primarne intestinalne limfangiektazije kod odraslih – dijagnostički i terapijski izazov

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Abstract

Introduction. Primary intestinal lymphangiectasia is a rare disorder, characterized by abnormal dilation of intestinal lymphatic vessels and extensive enteric loss of lymph rich in plasma proteins, lymphocytes and chylomicrons. The main characteristics of the disease are hypoalbuminemia, hypogammaglobulinemia, lymphocytopenia, and more rarely, the deficit of liposoluble vitamins and anemia. Except for primary, there are secondary lymphangiectasia, associated with celiac disease, malignant, infective and inflammatory diseases of the small intestine, fibrosis, liver and cardiovascular diseases. **Case report**. A male, 33 years of age, presented for his medical examination suffering from diarrhea and edema. The diagnosis was established upon the histological examination of a small intestine biopsy during double balloon enteroscopy, which revealed changes only in one segment of the intestine examined. Such a finding was later confirmed by the video endoscopy capsule. **Conclusion**. The diagnosis of intestinal lymphangiectasia is usually established before the age of 3, but it can also be diagnosed in adults. The diagnosis is based on the histological analysis of the intestinal mucosa biopsy, obtained by endoscopic procedures. The diagnosis of primary intestinal lymphangiectasia is also made upon the exclusion of secondary causes.

Key words:
diarrhea; lympangiectasis; hypoalbuminemia; lymphopenia; protein-losing enteropathies.

Apstrakt

Uvod. Primarne intestinalne limfangiektazije su retko oboljenje koje karakterišu abnormalno dilatirani crevni limfni sudovi i ekstenzivan enterični gubitak limfe koja je bogata proteinima plazme, limfocitima i hilomikronima. Osnovne karakteristike oboljenja su hipoalbuminemija, hipogamaglobulinemija, limfocitopenija, ređe deficit liposolubilnih vitamina i anemija. Osim primarne, limfangiektazije mogu biti i sekundarne, u sklopu celijakije, malignih, infektivnih i zapaljenskih bolesti tankog creva, fibroze, bolesti jetre i kardiovaskularnih oboljenja. **Prikaz bolesnika**. Muškarac, star 33 godine, javio se na pregled sa tegobama u vidu proliva i edema potkolenica. Dijagnoza je postavljena patohistološkim pregledom biopsija tankog creva pri *double balloon* enteroskopiji gde su promene viđene samo u jednom segmentu pregledanog creva, što je kasnije potvrđeno i videoendoskopskom kapsulom. **Zaključak**. Dijagnoza intestinalnih limafangiektazija postavlja se uglavnom pre treće godine života, ali može se dijagnostikovati i kod starijih osoba. Osnov dijagnoze je patohistološka analiza bioptata sluznice creva dobijenih endoskopskim procedurama. Dijagnoza primarnih intestinalnih limfangiektazija se postavlja i isključivanjem sekundarnih uzroka.

Ključne reči:
dijareja; limfangiektazija; hipoalbuminemija; limfocitopenija; enteropatije sa gubitkom proteina.

Introduction

Primary intestinal lymphangiectasia (PIL) is characterized by abnormally dilated intestinal lymphatic vessels and an extensive enteric loss of lymph rich in plasma proteins, lymphocytes and chylomicrons. The disorder was first described by Waldmann et al. [1] in 1961. The main characteristics of the disease are hypoalbuminemia, hypogammaglobulinemia, lymphocytopenia, and more rarely, the deficiency of liposoluble vitamins and anemia. Except for primary, lymphangiectasia can

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be secondary, as a consequence of lymphoma, mesenteric tuberculosis and sarcoidosis, Crohn's disease, Whipple's disease, celiac disease, retroperitoneal carcinoma or fibrosis, chronic pancreatitis, scleroderma, systemic lupus erythematosus, rheumatoid arthritis, congestive heart failure, constrictive pericarditis, Budd-Chiari's syndrome, sclerosing mesenteritis and intestinal endometriosis. The prevalence of the disease is not known, and thus far approximately 300 cases have been described in the literature [2, 3]. In most cases, it is diagnosed between ages 3 and 10 (being rare among adolescents and adults) [4]. The precise etiology is unknown, but it is considered to be related to the change in the regulatory molecules involved in the lymphangiogenesis in the intestinal mucosa, with the consequent lymphatic hypoplasia, lymph flow obstruction, which leads to the elevated pressure in the lymphatic vessels, their dilation, rupture and lymph leakage into the intestine lumen. The disease is clinically manifested with edemas in lower extremities, less frequently in the face or scrotum, effusions (pleural, pericardial, ascites), diarrhea or subocclusive intestinal disorders, rarely with anemia, fat and liposoluble vitamin malabsorption. Intestinal lymphangiectasia (IL) is diagnosed on the basis of the histological analysis of intestinal mucosa. The diagnosis of PIL is also made upon the exclusion of secondary causes [5].

Case report

A 33 years-old patient, was hospitalized under suspicion of nephrotic syndrome, with lower limb swelling, moderate diarrhea, with general weakness and fatigue. He had no history of previous disease or relevant hereditary diseases, occasionally consumed alcohol and was a smoker. The objective examination revealed that the patient's general condition was good; Body mass index (BMI) was 19.6 kg/m^2 (normal range (nr): 18.5–25 kg/m^2), with prominent edemas in the lower limbs and normal findings in the organ systems. The laboratory findings indicated marked hypoproteinemia [(29 g/L (nr 60–80 g/L)], hypoalbuminemia [(15.4 g/L (nr 35–50 g/L)], with low immunoglobulin levels IgG 2.04 g/L (nr 6.1–16 g/L) and IgM 0.25 g/L (nr 0.4–2.3 g/L), while the values of IgE and IgA are normal. Proteinuria was not within the nephrotic range (24h-proteinuria 239 mg/diuresis). The count of leukocytes was normal, but with the presence of lymphocytopenia [0.36 $\times 10^9$/L (nr) 2–8 $\times$ 10^9/L)]. The parameters of renal and liver functions and the acute phase reactants were within the reference range. Tissue transglutaminase IgA antibody levels were negative and so were the HBsAg, anti HCV and anti HIV. Abdominal ultrasound examination revealed ascites. After the systemic disease of connective tissue was excluded, the suspicion of protein-losing enteropathy was assumed. Upper endoscopy showed the mucous membrane of the descending duodenum which was lightly edematous, whereas the histological finding corresponded to the chronic enteritis without atrophy PAS positive macrophages and polymorphonuclears. Ileocolonoscopy and histological findings are normal and the computed tomography (CT) scan of the abdomen showed a lightly dilated small intestine, with the diffuse edematous wall, without lymphadenomegaly, thrombosis, or changes in the retroperitoneum. Enteroclysis revealed the jejunum with partially thickened folds, and the double balloon (DB) enteroscopy of the proximal jejunum detected several polypoid changes 6–8 mm in diameter (single and clustered) in the bowel segment approximately 15 cm long (Figure 1). The histological examination detected intestinal villi of regular height, some of which were dilated due to enlarged lymph ducts. In the lamina propria there were slightly to significantly enlarged lymph ducts with slight infiltration of lymphocytes, plasmocytes and a lesser count of eosinophils. This finding corresponds to chronic enteritis with lymphangiectasia (Figure 2). In order to evaluate the extent of the changes in the small intestine, video endoscopy capsule examination was performed and it revealed polypoid changes with sporadic white spots, arranged in groups (Figure 3). In view of the histological findings and the patient's age, further examinations were performed in order to exclude secondary causes of IL. Echocardiography excluded congestive heart failure and constrictive pericarditis, and negative angiotensin-converting enzyme (ACE) and purified protein derivative (PPD) tests, as well as subsequent *Mycobacterium tubercu-*

Fig. 1 – Double ballon enteroscopy of the proximal jejunum demonstrating polypoid changes 6–8 mm in diameter with white spots.

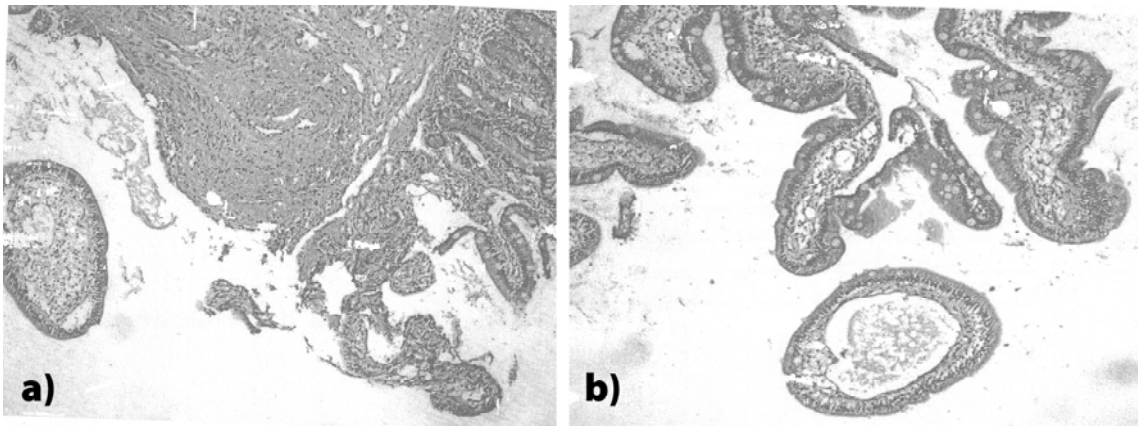

Fig. 2 – Histopathological finding reveals intestinal willi of regular height with dilated lymph ducts and infiltration of lymphocytes, plasmocytes and eosinophils in *lamina propria*: a) hematoxylin and eosin, × 50; b) hematoxylin and eosin, × 200.

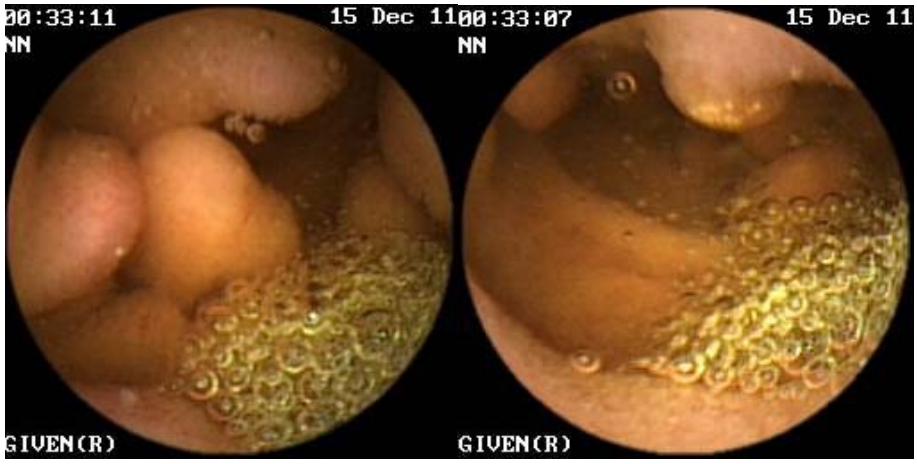

Fig. 3 – Video endoscopy capsule examination reveals polypoid changes with sporadic white spots, arranged in groups.

lose test excluded sarcoidosis and tuberculosis. The bacteriological stool test excluded an infective etiology. The diagnosis of PIL was made on the basis of typical clinical, endoscopic findings and histological confirmation with laboratory findings of lymphocytopenia, hypoalbuminemia, hypogammaglobulinemia and deficiency of proteins (transferrin, ceruloplasmin, fibrinogen), after the exclusion of potential causes of secondary lymphangiectasia. The treatment recommended a diet with a limited intake of fats with the use of medium-chain fatty acids. The hypoalbuminemia was treated with the administration of parenteral albumin preparations. In the subsequent course of the disease the patient did not consistently comply with the dietary recommendations for financial reasons, and as a result was occasionally hospitalized for parenteral albumin administration.

Disscusion

IL is characterized by lymph and lymphocyte leakage into the small intestine lumen, due to the dilation of the lymph vessels of the intestinal wall and mesentery. It can be classified into primary and secondary. PIL is a rare disease with approximately 300 cases described in the literature [6]. The prevalence is unknown and is increasing with the introduction of endoscopic video capsule and enteroscopy diagnostic procedures. There is no difference in prevalence between gender, and is most commonly diagnosed between ages 3 and 10, and rarely among adolescents and adults [4]. Secondary lymphangiectasia develops as the consequence of lymph flow obstruction caused by other diseases. Our patient was diagnosed with PIL upon the exclusion of secondary causes of lymphangiectasia (celiac disease, malignant diseases, infective or inflammatory diseases, fibrosis, liver or cardiovascular diseases). The disease is clinically manifested with edemas, effusions (pleural, pericardial, *ascites*), diarrhea, rarely with obstructive ileus, anemia, malabsorption of fats and liposoluble vitamins.

Alpha-1 antitrypsin is a protein of molecular mass similar to albumin, and alpha-1 antitrypsin clearance is used for the confirmation of protein-losing enteropathy. Unfortunately, this diagnostic procedure was not available to us, so upon the exclusion of other causes of hypoalbuminemia and hypogammaglobulinemia, it was assumed that the disease involved was a protein-losing enteropathy. The loss of certain proteins and their levels in the serum can vary significantly. The lowest concentrations

are found in serum proteins with long half-life and slow synthesis, so the levels of IgG, IgM and IgA will be significantly reduced, while insulin and IgE will be normal due to their short half-life. Lymphocytopenia is a finding that suggests IL and is not found in other protein-losing enteropathies [7, 8]. PIL is not easily diagnosed due to localization of changes in the small intestine, which is the most difficult to access in endoscopic diagnostics. The detection of lesions by upper endoscopy and ileocolonoscopy is limited, while radiologic examination cannot confirm the diagnosis. The diagnosis is made on the basis of the histological examination of the intestine biopsy obtained with DB enteroscopy or the intestine resectate examination. Video endoscopic capsule is more frequently used to detect the disease, and also enabling to determine its extent [9, 10]. In our case the diagnosis was made by small intestine biopsy taken of DB enteroscopy, where the changes were seen in one segment of the intestine examined, and the disease, localized in a single segment, was subsequently confirmed by video endoscopic capsule.

The main method recommended to treat PIL is diet with the reduced intake of fats containing long-chain fatty acids, since they turn into chylomicrons, leading to the obstruction of lymph vessels, elevated lymphatic pressure and lymph leakage. The consumption of medium-chain fatty acids (MCT) is recommended, since they are absorbed directly into the portal blood flow [11–13], resulting in the bypass of the lymphatic flow. By analyzing the literature and individual case reports (55 cases extracted), Desai et al. [14] examined the influence of MCT diet in PIL therapy in pediatric population. They examined the outcome of the disease in 27 patients who were on MCT diet, out of which 63% had complete resolution of symptoms, in contrast to the group of 28 patients who were not on the diet and where only 35.7% showed complete resolution [14]. Unfortunately, there is no curative or standard treatment of PIL. In several cases the beneficial effect of octreotide and is described in patients with diarrhea refractory to diet [11]. Although the mechanism of the action is still not clear, it is believed that octreotide significantly reduces splanchnic blood flow, thus reducing the intestinal absorption of fats. However, it does not have a significant effect on hypoalmuminemia, since thus diarrhea recurs after the discontinuation of the medication [11, 15]. The usage of antiplasmins in certain cases led to the increase in the level of T lymphocytes and serum immunoglobulins, and even the withdrawal of endoscopic changes, while corticosteroids were used with varying success [16]. In the localized forms of the disease refractory to diet and medications, as an alternative, surgical procedures were successfully performed [17]. Our patient's clinical and laboratory improvement occurred after the application of dietary therapy and the parenteral albumin substitution.

PIL patients, in view of the lymphocyte loss, especially CD4+, develop a disposition to virus skin warts and B cell lymphomas, which increases with the duration of the disease [9]. It is still not clear whether the occurrence of B lymphoma is accidental or if it is related to PIL. Five percent of patients in whom the disease persists over 30 years develop lymphoma that can be limited to the gastrointestinal tract, where the disease is localized, or can be extraintestinal. Although they have severe hypogamaglobulinemia and lymphopenia, the risk of the emergence of pyogenic bacterial and opportunistic infections is not significantly increased [18, 19].

Conclusion

Primary intestinal lymphangiectasia is a rare disease which usually requires extensive diagnostics, especially in adult patients, in whom the diagnosis is made upon the exclusion of secondary causes of lymphangiectasia. In protein-losing enteropathy with lymphocytopenia, intestinal lymphangiectases should always be suspected, and regardless of the patient's age, the possibility of primary intestinal lymphangiectasia should not be discarded. Despite the constant new findings about this disease, no significant advancement in the therapy of primary intestinal lymphangiectasia has be made. In the majority of patients medium-chain fatty acids diet is successfully applied to control the disease, while recurrence and complications are possible if there is no adequate compliance.

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CASE REPORT

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Extremely tortuous coronary arteries – When optical coherence tomography and fractional flow reserve did not help us much

Ekstremno tortuozne koronarne arterije – kada optička koherentna tomografija i frakciona rezerva protoka ne pomažu mnogo

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Abstract

Introduction. Extreme coronary tortuosity may lead to flow alteration resulting in a reduction in coronary pressure distal to the tortuous segment, subsequently leading to ischemia. Therefore the detection of a true cause of ischemia, i.e. whether a fixed stenosis or tortuosity by itself is responsible for its creation, with non-invasive and invasive methods is a real challenge. **Case report**. We presented a case of a patient with a history of stable angina [Canadian Cardiovascular Society (CCS class II)], an abnormal stress test and coronary tortuosity without hemodynamically significant stenosis. Due to suspected linear lesion between the two bends in proximal segment of Right coronary artery (RCA) we performed optical coherence tomography (OCT), minimum lumen area (MLA)-13.19 mm2) and fractional flow reserve (FFR) RCA (0.94). We opted for conservative treatment for stable angina. **Conclusion**. When tortuosities are associated with atherosclerosis in coronary artery for determination of true cause of ischemia invasive methods can be used, such as OCT and FFR.

Key words:
coronary vessels; angina pectoris; blood flow velocity; coronary angiography; tomography, optical coherence.

Apstrakt

Uvod. Ekstremni tortuozitet koronarne arterije može izazvati promene u protoku krvi i samim tim dovesti do pada koronarnog pritiska distalno od tortuoznog segmenta što bi moglo da se manifestuje ishemijom. Samim tim, otkrivanje uzroka ishemije, odgovornog za njen nastanak, šta je fiksna stenoza ili sam tortuozitet, kako neinvazivnim tako i invazivnim metodama, predstavlja pravi izazov. **Prikaz bolesnika**. Prikazan je bolesnik sa stabilnom anginom pektoris [*Canadian Cardiovascular Society* (CCS II)], pozitivnim stres testom i ekstremno tortuoznim koronarnim arterijama, međutim, bez hemodinamski značajnih stenoza. Usled suspektne linearne lezije između dve krivine na proksimalnom segmentu desne koronarne arterije urađena je optička koherentna tomografija (OCT) minimum lumen area (MLA) -13,19 mm^2) i *fractional flow reserve* (FFR) (0,94) pomenute arterije. Opredelili smo se za konzervativno lečenje stabilne angine pektoris. **Zaključak**. Kod udružene ateroskleroze i tortuoziteta koronarne arterije, za ispitivanje uzroka ishemije mogu se koristiti neke od invazivnih metoda, kao što su OCT i FFR.

Ključne reči:
koronarni krvni sudovi; angina pektoris; krv, brzina protoka; angiografija koronarnih arterija; tomografija, optička, koherentna.

Introduction

Extreme coronary tortuosity is conventionally defined as two or more consecutive 180° turns in a major epicardial artery assessed by visual estimation [1]. The etiology of arterial tortuosity is still unclear, but it is believed to be caused by age-dependent or pathological changes of the elastic material in the vessels [2]. We presented a case of a patient with anginal complaints, positive stress test results and extreme tortuous arteries. In this clinical setting, without presence of obvious significant coronary stenosis, it is challenging to determine true origin of anginal complaints.

Case report

A 63-year-old man was admitted at the Cardiology Department for an elective coronary angiography. He had a previous history of anginal complaints [Canadian Cardiovascular Society (CCS II)]. Electrocardiogram showed incom-

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plete right bundle branch block and T wave inversion in III lead. Risk factors for ischemic heart disease were hypertension, hyperlipoproteinemia and smoking. Echocardiography revealed eccentric hypertrophy of the left ventricle with septal hypokinesia and EF 50–55%. Exercise stress test on moderate exertion showed marked, ischemic ST depressions in inferior leads. Coronary angiography was performed through radial artery despite severe tortuosity of radial and subclavian artery, combined with radial spasm (Figure 1). Coronarography revealed absence of the left main (LM) i.e. separate ostiums of left anterior descending (LAD) and left circumfex artery (LCx.) LAD was tortuous artery, without angiographically significant stenosis, and also irrigated big part of an inferior wall (Figure 2). LCx was extremely tortuous artery, with mild atherosclerosis (Figure 2). Right coronary artery (RCA) was extremely tortuous artery proximally, with suspected linear lesion between the two bends (Figure 3). Thus, a decision was taken to further examine the proximal segment of the RCA with optical coherence tomography (OCT) and fractional left main flow reserve OCT, and FFR RCA was performed through femoral artery. First ASAHI SION blue guide wire was easily placed down in postero-lateral (PL) branch. Than OCT RCA revealed only mild atherosclerosis minumum lumen area (MLA) measured in the lesion level-13.19 mm^2], with an oval cross section, typical for tortuous arteries [9] (Figure 4). It turned out that the so-called lesion is nothing more than a curve on an artery. FFR RCA, measured with dedicated pressure wire

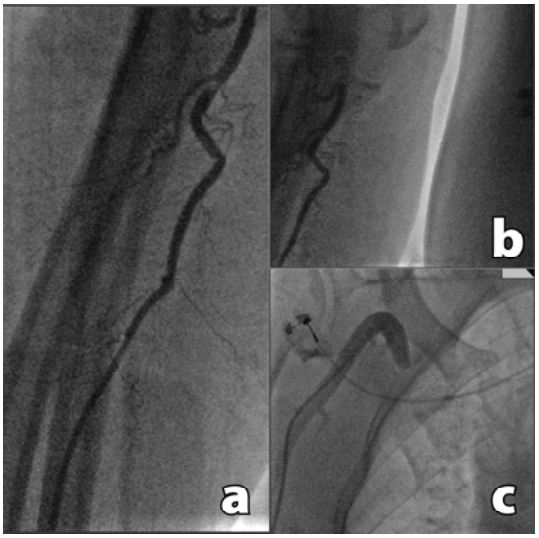

Fig. 1 – a) Spasm; b) Severe tortuosity of radial artery, c) Severe tortuosity of subclavian artery.

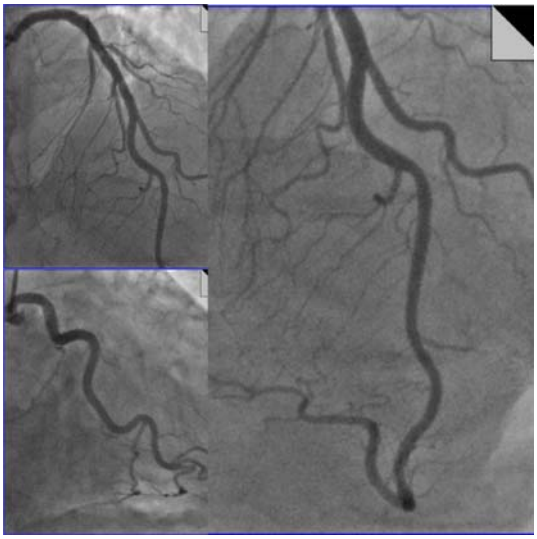

Fig. 2 – Left anterior descending (LAD): tortuous artery, without significant stenosis, also irrigates big part of an inferior wall of left circumflex artery (LCx): extremely tortuous artery, with mild atherosclerosis.

Fig. 3 – Right coronary artery (RCA): extremely tortuous artery proximally, suspected linear lesion proximally, between the two bends.

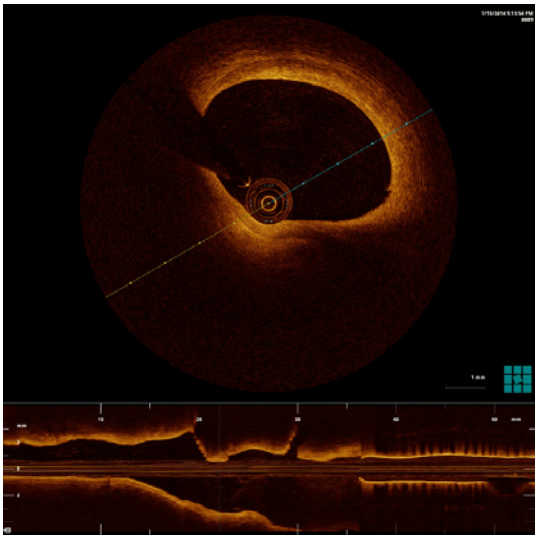

Fig. 4 – Optical coherence tomography (OCT) of right coronary artery (RCA) reveals only mild atherosclerosis, with an oval cross section in the lesion level, typical for tortuous arteries [minimum lumen area (MLA) - 13.19 mm^2]. It turned out that the so-called lesion is nothing more than a curve on an artery.

medially, showed a clearly negative result – 0.94 (Figure 5). RCA angiography with Amplatz GC showed partial straightening of the curves with pressure wire and OCT (Figure 6). Thus, we opted for the optimal medical therapy for treatment stable angina.

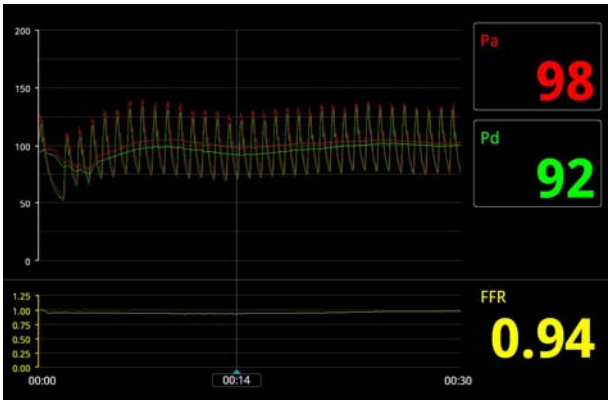

Fig. 5 – Fractional flow reserve (FFR) of right coronary artery (RCA) showed a clearly negative result (0.94), measured medially.

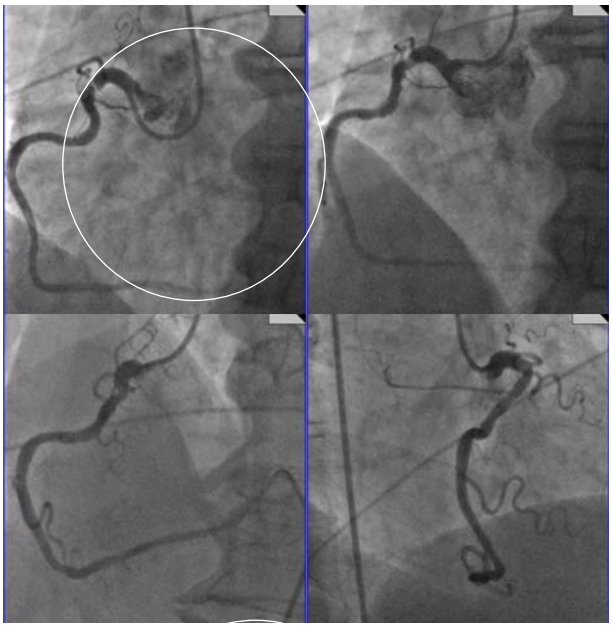

Fig. 6 – Right coronary artery (RCA) angiography with Amplatz GC, partial straightening of the curves with pressure wire and optical coherence tomography (OCT) catheter.

Disscusion

Determinants of coronary tortuosity are gender, age, left ventricle (LV) volume, and muscle mass [3]. Coronary artery tortuosity often correlates with systemic artery tortuosity [4]. Tortuosity is more often seen in the atherosclerotic arteries than in other arteries [5], but less frequently in those with hemodynamically significant stenosis [1]. It is hypothesized that coronary tortuosity leads to flow alteration resulting in a reduction in coronary pressure distal to the tortuous segment of the coronary artery, subsequently leading to ischemia [6–8]. There might be a compensatory mechanism of the tortuous coronary system which will compensate for the theoretical decrease in perfusion pressure in coronary tortuosity at rest [6, 9], while during exercise lack in ability to maintain adequate blood supply [5]. In our case we had a patient with a history of anginal complaints, an abnormal stress test and coronary tortuosity with suspected hemodynamically significant stenosis shown on coronarography. In situation when exercise stress test indicated that inferior wall was in induced ischemia and we found suspected linear lesion between the two bends on proximal segment of RCA, we could not expect that some otherstress tests [stress echo, single/photom emission computed tomography (SPECT)…] would show us what was the real cause of ischemia (stenosis of tortuosity by itself). That is why we performed OCT and FFR RCA, which eventually ended up negative (there was no lesion, nor ischemia). However, it is noticeable that during the performance of FFR, as well as during OCT, came to a partial straightening of the curves (Figure 6), which lead us to accept the result with an amount of doubt. Also there is a myocardial hypertrophy so, even after 100 μg of adenosine was given as intracoronary bolus we cannot be sure that hyperemia was achieved. Conservative treatment was chosen for the treatment of stable angina.

Conclusion

When extreme coronary tortuosity is present, there is also a stenosis of the artery, and it could be very difficult to determine the true cause of ischemia by non-invasive test (i.e. whether it comes from a stenosis or from tortuosity by itself). Further invasive examination [(OCT, FFR, intravascular ultrasound (IVUS)], although quite challenging to perform, could help to determine the hemodynamically significance of stenosis and thus define the manner of treatment.

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Neuromyelitis optica spectrum disorder in patient with systemic lupus erythematosus – our experience

Bolesti spektra optičkog neuromijelitisa kod bolesnice sa sistemskim eritemskim lupusom – naše iskustvo

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Abstract

Introduction. Neuromyelitis optica spectrum disorder (NMOSD) is a rare demyelinating immune-mediated central nervous system disease. It is extremely rare to occur in patients with systemic lupus erythematosus (SLE), and it represents a diagnostic and therapeutic challenge. **Case report.** A 38-year-old Caucasian woman with medical history of SLE and new onset of flaccid paraparesis, fecal and urinary incontinence, persistent nausea and vomiting was admitted to our hospital. Based on the clinical presentation, magnetic resonance imaging findings and positive aquaporin 4 (AQP4) antibodies, a NMOSD with coexisting SLE were diagnosed. Pulse-doses of cyclophosphamide and glucocorticoids were efficient in patient treatment. **Conclusion.** In a patient with SLE and symptoms of longitudinal extensive transverse myelitis and/or optic neuritis and area postrema syndrome, assessment of AQP4 antibodies is neccessary for diagnosing NMOSD. Accurate diagnosis, and timely and long-term administration of immunosuppressive therapy are crucial for favorable outcome of these two coexisting diseases.

Key words:
lupus erithematosus, systemic; neuromyelitis optica; diagnosis; aquaporin-4; antibodies; drug therapy; treatment outcome.

Apstrakt

Uvod. Bolesti iz spektra optičkog neuromijelitisa (BSON) su retka imunski - posredovana demijelinizaciona oboljenja centralnog nervnog sistema. Kako se veoma retko javljaju kod bolesnika sa sistemskim eritemskim lupusom, predstavljaju veliki dijagnostički i terapijski izazov. **Prikaz bolesnika.** Bolesnica, stara 38-godina, sa sistemskim eritemskim lupusom i novonastalom flakcidnom paraparezom, inkontinencijom urinarnog i analnog sfinktera, prolongiranom mučninom i povraćanjem, primljena je u našu kliniku. Na osnovu kliničke prezentacije, nalaza nuklearne magnetne rezonance i pozitivnih antitela prema akvaporinu 4 (AQP4) dijagnostikovana je BSON udružena sa sistemskim eritemskim lupusom. Terapija pulsnim dozama ciklofosfamida i glikokortikoida je bila efikasna. **Zaključak.** Kod bolesnika sa sistemskim eritemskim lupusom i simptomima longitudinalnog ekstenzivnog tranverzalnog mijelitisa i/ili optičkog neuritisa i sindroma *area postrema*, neophodno je odrediti AQP4 antitela radi postavljanja dijagnoze BSON. Tačna dijagnoza, pravovremena i dugotrajna primena imunosupresivne terapije je od presudnog značaja za povoljan ishod udruženosti ove dve bolesti.

Ključne reči:
lupus, eritematozni, sistemski; neuromijelitis optika; dijagnoza; akvaporin-4; antitela; lečenje lekovima; lečenje, ishod.

Introduction

Neuromyelitis optica (NMO) was described at the end of 19th century by Eugene Devic, and until recently it has been commonly called the Devic's syndrome [1]. Traditionally it was defined as a monophasic demyelinating disease of central nervous system (CNS) consisting of optic neuritis (ON) and transverse myelitis (TM). It has changed relatively recently, first with the discovery of a highly specific serum aquaporin-4 (AQP4) immunoglobulin G antibody [2], then,

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with an understanding that affection of central nervous system (CNS) in this disease may be more restricted, or more extensive than previously thought. In turn, this led to understanding that all demyelinating diseases with positive AQP4 antibodies and variable affection of CNS should be labeled as NMO spectrum disorders (NMOSD) [3]. Current diagnostic criteria also recognise monophasic and relapsing NMOSD, as well as AQP4 antibody positive and AQP4 antibody negative NMOSD, both having characteristic magnetic resonance imaging (MRI) findings in one of six regions of CNS (optic nerve, spinal cord, area postrema, brainstem, diencephalon and cerebelum) [4].

It is not so uncommon that a patient with NMOSD develops another autoimune disorder, such as systemic lupus erythematosus (SLE), Sjögren's syndrome, autoimmune thyreoiditis, or myasthenia gravis [5]. On the other side, it is estimated that the probability of a patient with SLE developing NMO is extremely low – approximately 1 : 5.000.000 [6]. Neurological manifestations similar to NMO and due to CNS demyelinisation can occur as a part of clinical picture of SLE. It is therefore important to distinguish if the neurological manifestations are caused by autoimune disease, or, they are just a part of coexisting NMOSD.

Case report

A 38-year-old Caucasian woman with a medical history of SLE was transferred to our hospital due to longitudinally extensive transverse myelitis (LETM) and for further diagnostics and treatment.

SLE was diagnosed a year prior to this, and was based on nonerosive polyarthritis, leukolymphopenia, positive antinuclear antibodies (ANA) and antibodies to double- stranded DNA (anti-dsDNA). Since then, the patient had been treated with prednisolone 10 mg/day and hydroxychloroquine 400 mg/day. After introduction of the therapy the patient was generally well with the exception of periodical polyarthralgia.

Three weeks before admission to our institution, a fever, persistant nausea, vomiting, hiccups, headache, abdominal pain and dry cough occurred. She was admitted to a local hospital and initially treated for gallbladder calculosis and cholecystitis. During the second week in the hospital, she became somnolent, developed a rapidly progressive paraparesis (within 24 hours) and fecal and urinary incontinence. MRI scanning of the cervical and thoracic spine showed hyperintense signal in T2-weighted sequence in C7-T1, T1-T4 and T8-T11 spinal cord segments (Figure 1). MRI scanning of the brain showed hyperintense signal in T2-weighted sequence in dorsal and lateral medulla on the left (Figure 2). Postcontrast signal enhancement in both optical nerves was observed. The NMO was suspected. The patient was treated with pulse doses of methylprednisolone 1 gram daily for 5 days followed by intravenous immunoglobuline (IVIg) 400 mg/kg day for 4 days, while hydroxychloroquine was discontinued. Patient's state was deteriorating despite the treatment, so she was transferred to our hospital.

Fig. 1 – T2W magnetic resonance imaging (MRI, sagital section) of cervical and thoracic spine showing hyperintense signal in C7-T1, T1-T4 and T8-T11 spinal cord segments (arrow).

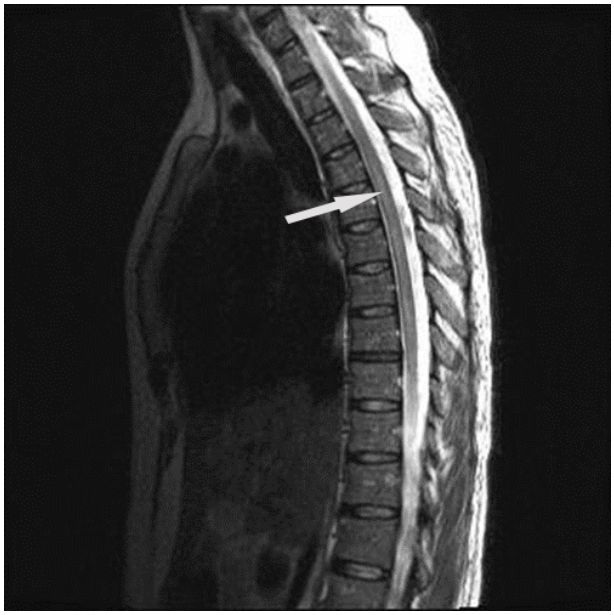

Fig. 2 – T2W magnetic resonance imaging (MRI, axial section) of brain showing hyperintense signal in dorsal and lateral medulla oblongata on the left (arrow).

On admission, she had a low grade fever (37.8^{o}C), mild malar rash and low blood pressure (80/60 mmHg). Basal respirations were auscultatory inaudible. Flaccid paraplegia and spastic paresis of both arms as well as urinary retention and fecal incontinence were the dominant neurological findings upon admission. There was also a discrete right peripheral *n. facialis* weakness, billateral hand action tremor, mild impa-

irment of light touch, pinprick and vibration sensation in the legs, but without a clear sensory level. Expanded disability severity scale (EDSS) at this point was 8.5.

Blood tests showed erythrocyte sedimentation rate (ESR) of 96 mm/h, C-reactive protein (CRP) of 29.63 mg/L (normal range: < 5 mg/L), leukocytes 2.7×10^9/L (normal range 4-10 $\times 10^9$/L), hemoglobin 74 g/L (normal range: 130–180 g/L). Serology testing showed positive ANA 1 : 160, anti dsDNA 167 (normal range: 0–25) and positive direct Coombs test, polyclonal hypergammaglobulinemia IgG of 18 g/L (normal range: 5–16 g/L), low C3 and C4. Serum AQP4 antibody (indirect immunofluorescence test) was 1 : 2,560 (normal range < 1 : 10). Anticardiolipin antibodies, lupus anticoagulant and anti-Smith antibody were negative.

Cerebrospinal fluid analysis showed elevated level of protein 0.69 g/L (normal range < 0.45 g/L), lowered glucose 1.9 mmol/L (normal range: 2.2–4.6 mmol/L), elevated albumin index 11.23 (normal range < 5.7), without pleocytosis and with normal IgG index. Olygoclonal bands, *Borrelia*, neurotropic viruses and acid-resistant bacillus were negative.

Computed tomography of the chest showed bilateral pleural effusions.

Visual evoked potentials (VEP) showed prolonged P100 latencies of 127 ms on the left and 121 ms on the right eye. The patient did not report any visual loss, nor could any visual disturbance be observed through usual clinical examination.

Based on the presence of longitudinally extensive transverse myelitis (LETM), subclinical ON, area postrema syndrome and positive AQP4 antibodies, NMOSD were diagnosed. SLE erythematosus disease activity index 2,000 (SLEDAI-2K) was at this point 9.

Monthly cyclophosphamide (CF) pulses of 15 mg/kg were introduced, and methylprednisolone 1 mg/kg was continued for 2 weeks, then, gradually reduced. Followed by therapy introduction the neurological symptoms gradually improved and sphincter function completely recovered.

Over the next 6 months the patient received 6 monthly pulse doses of CF and methylprednisolone dose was 10 mg/day. On follow-up after 6 months, she was ambulatory without any assistance on distances greater than 1,000 m. Neurological examination revealed residual spastic paraparesis to a lesser degree, with EDSS of 3.5. SLE activity was low (SLEDAI-2K: 3).

Discussion

In the SLE patient, according to American College of Rheumatology nomenclature, 19 neuropsychiatric syndromes (NPSLE) can be defined [7]. Among the least frequent is myelitis, present in 1–2% of patients [8]. It can present itself as TM consisting of spinal cord lesions of one to two spinal segments or as LETM extending 3 or more vertebral segments confirmed by T2-weighted MRI [9].

The relationship between TM and present antiphospholipid (APL) antibodies in SLE is proven. It is considered that APL-induced vasculitis of spinal blood vessels and direct cytotoxic effect to nervous tissue have a role in occurrence of TM in patients with SLE [10].

Since LETM represents a very rare manifestation of SLE, other possible causes should be sought for, including multiple sclerosis, infections, tumors, trauma and nutritional deficiencies. LETM is also one of the main clinical characteristics and a part of the modern diagnostic criteria for NMOSD [11]. In our patient, repeated assessment of antiphospholipid antibodies was negative, possible infective causes were excluded and McDonald's revided 2010 criteria for multiple sclerosis were not met [12]. VEP latencies are often delayed in NMOSD (as it was in this case), but this finding, although illustrative of optical nerve demyelination is not specific for NMOSD, and can be seen in a variety of clinical entities, especially in multiple sclerosis [13]. In our case, there were several more unique clinical characteristics of the NMOSD – constant hiccups, nausea and vomiting. These are manifested in 15.7–62% of patients [14]. Area postrema and solitary tract nuclei in dorsomedial medulla are frequently affected areas (as confirmed in the presented case by MRI finding), which can be explained by a rich expression of APQ4 in this area [15].

So far, the interconnection between autoimmune diseases and NMOSD has not been completely clarified. It is considered that there is a genetic predisposition of certain patients for developing several types of autoimmune diseases. It has been demonstarted that only patients with SLE who have ON and/or myelitis have positive APQ4 antibodies, whereas those with other NPSLE do not have [16].

In the NMOSD treatment, pulse-dose corticosteroids, plasmapheresis and IVIgs are recommended in acute phase. This is followed by long-term immunopression with rituximab, azathioprine and mycophenolate mofetil as recommended [17]. There are no recommendations for the treatment of patients with coexisting SLE and NMOSD. Current knowledge is based on retrospective cases and short series [18].

Conclusion

Antiaquaporin-4 antibody should be sought for in patients with SLE and the first attack of LETM, ON and/or area postrema syndrome. Positive anti aquaporin-4 antibodies in these cases can easily lead to diagnosing a coexisting NMOSD in patient with SLE.

Accurate and timely diagnosis followed by a long-term immunosupressive therapy are necessary to reduce neurological sequellae and establish adequate control of both diseases.

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I N M E M O R I A M

prof. dr DRAGAN STUPAR
pukovnik u penziji
(1937–2017)

Dana 3. oktobra 2017. godine okupili smo se na Farmaceutskom fakultetu Univerziteta u Beogradu kako bismo se prisetili života i dela našeg uvaženog profesora, kolege i prijatelja, profesora Dragana Stupara. O radu i liku profesora Stupara govorili su general-major u penziji, primarijus dr Branislav Popović (nekadašnji načelnik Sanitetske uprave), prof. dr Vesna Matović (predsednica Saveza farmaceutskih udruženja Srbije), prof. dr Svetlana Ibrić, prof. dr Marija Primorac, prof. dr Ljiljana Tasić i prof. dr Dušanka Krajnović sa Farmaceutskog fakulteta Univerziteta u Beogradu.

Profesor Dragan Stupar ostavio nas je zauvek 30. jula 2017. godine, tiho i dostojanstveno, kako je i živeo. Otišao je iznenada, tako da smo mi koji smo ga poznavali i profesionalno sarađivali sa njim sa velikom tugom i nevericom primili vest da je preminuo. I danas pokušavamo da tu prazninu koju osećamo ispunimo sitnim fragmentima iz života i sećanja na vreme provedeno sa njim. Ovu duboku prazninu ispunjavaju skoro 20 godina zajedničkog rada od prijema na posao do poslednjeg zajedničkog diplomskog ispita.

Profesor je bio više od mentora, pedagoga, učitelja. Ličnim primerom pokazivao je kako treba graditi mrežu saradnika i istraživača, okupljajući oko sebe svršene studente „svoje diplomce“ koji su radove prezentovali na naučnim i međunarodnim skupovima pod budnim nadzorom i uz mentorstvo profesora, i koji su ostajali u trajnim profesionalnim vezama sa njim do kraja života.

Profesor Stupar rođen je 17. jula 1937. godine u Rasavcima kod Prijedora, u Bosni i Hercegovini. Osnovnu školu pohađao je u Sanskom Mostu i Oštroj Luci, a gimnaziju u Pančevu, Prijedoru i Novom Sadu. Godine 1958. završio je Sanitetsku oficirsku školu u Ljubljani, a Farmaceutski fakultet Univerziteta u Beogradu okončao je 1965. godine. Deset godina kasnije na istom fakultetu odbranio je i doktorsku tezu pod naslovom „Vojna farmacija u Srbiji u XIX veku“. Profesor je aktivno radio na Vojnomedicinskoj akademiji (VMA) u Beogradu, gde je 1981. god. izabran u zvanje docenta, potom 1985. godine za vanrednog profesora. Bio je rukovodilac nastave za farmaceute u Školi rezervnih oficira saniteta, a ostaće u sećanjima mnogih generacija vojnih lekara, stomatologa i farmaceuta, koje je učio organizaciji sanitetskog snabdevanja i organizaciji farmaceutske delatnosti u miru i ratu. Duži niz godina obavljao je funkciju sekretara Nastavno-naučnog veća VMA.

Profesor je izveo generacije studenata Farmaceutskog fakulteta u Beogradu kojima je predavao Istoriju farmacije i farmaceutsku etiku, ali i medicinskih fakulteta u Banjaluci i Novom Sadu na kojima su se školovali farmaceuti. Bio je rukovodilac magistarskih studija za istoriju zdravstvene kulture za koju je Farmaceutski fakultet bio matičan sve do 2005. godine, kada su nastojanjima pojedinih čelnika iz uprave Fakulteta ove studije ugašene, i nikada nisu prevedene u doktorski program, uprkos njegovom velikom angažovanju i uz veliko razočaranje i žaljenje nas, njegovih saradnika. Učestvovao je u dodiplomskoj i poslediplomskoj nastavi, i po odlasku u penziju aktivno je predavao sve do 2005. godine na svom fakultetu u Beogradu, kao i u dodiplomskoj nastavi na Odseku za farmaciju Medicinskog fakulteta Univerziteta u Novom Sadu od 2003. do 2010. godine i Medicinskom fakultetu u Banjaluci od 2000. do 2014. godine.

Po odlasku u penziju objavljivao je znatno manje, ali nije prestao sa radom na obradi prikupljene građe, uglavnom o radu vojnog saniteta. U tom periodu, realizovao je veći broj priloga u vidu odrednica za leksikone i enciklopedije od nacionalnog značaja, kako civilne tako i vojne. Prof. Stupar je bio poznat po svojim radovima i uspešnim izlaganjima na domaćim i međunarodnim skupovima u ovdašnjim naučnim krugovima, ali i u inostranstvu, naročito u zemljama Balkana i bivše Jugoslavije. Znatan broj istoričara farmacije s punim pravom ga je visoko cenio kao jednog od najistaknutijih farmakoistoričara sa prostora bivše Jugoslavije, a taj odavno zasluženi ugled navodio ga je da i prema sebi i svom radu bude strog kritičar, što je zahtevao i od svojih saradnika. U periodu preseljenja Farmaceutskog fakulteta u namenski podignutu zgradu 1991. godine, profesor je brinuo da i zbirka Muzeja za istoriju farmacije dobije zasluženo mesto, te je uglavnom njegovom zaslugom muzejska postavka dobila svoje prostorije, da bi kasnije bila i delimično restaurirana njegovim angažovanjem krajem devedesetih godina. Mlađe kolege sa Katedre za farmaceutsku tehnologiju i kozmetologiju podsticao je da se uključe u sređivanje zbirke i njenu obradu, a nekolicina nas prezentovala je o ovome i radove sa profesorom u koautorstvu. Radio je do pred kraj života, marljivo sakupljajući građu o vojnom sanitetu i mestu i ulozi farmaceuta u njemu, posebno osvetljavajući period Velikog rata, kome se uvek iznova vraćao sa velikim interesovanjem.

Prof. Stupar bio je član redakcije i jedan od autora četvorotomne edicije „Sanitetska služba u narodnooslobodilačkom ratu Jugoslavije 1941–1945“, koju je izdao 1989. godine Vojnoizdavački i novinski centar.

Vredno je spomenuti i njegovo angažovanje u brojim društvima i sekcijama posvećenim izučavanju istorije zdravstvene kulture, od kojih je u mnogima obavljao i funkciju sekretara ili predsednika u okviru Sekcije za istoriju farmacije Saveza farmaceutskih društava Jugoslavije, Saveza farmaceutskih udruženja Srbije, Farmaceutskog društva Makedonije, Sekcije za istoriju medicine i farmacije Srpskog lekarskog društva, Naučnog društva za istoriju zdravstvene kulture Srbije i Saveza naučnih društava za istoriju zdravstvene kulture Jugoslavije. Profesor je bio član Svetske farmaceutske federacije (FIP-a) u Akademskoj sekciji i Sekciji za istoriju farmacije. Njegovo angažovanje da Sekcija farmaceutskog društva Srbije postane ravnopravna članica Svetskog društva za istoriju farmacije urodilo je plodom 2003. godine, a zapažene su i njegove aktivnosti u mnogim profesionalnim asocijacijama, posebno u Farmaceutskom društvu Srbije (danas Savez farmaceutskih udruženja Srbije) i Farmaceutskoj Komori Srbije (FKS), čiji je bio počasni član. U teškim ratnim godinama, početkom devedesetih, profesor, je bio predsednik Farmaceutskog društva Srbije (1989-1993) i tada je uspeo da privremeno iseljeno Društvo sa celokupnom arhivom i bibliotekom smesti u prostorije Farmaceutskog fakulteta do iznalaženja rešenja za novi prostor. U prvom sazivu FKS, profesor je dao doprinos kroz rad u Komisiji za izradu etičkog kodeksa i profesionalnu etiku i kao zamenik predsednika Skupštine FKS.

Sve ovo ukratko navedeno ostaje kao trajno delo koje svedoči o ulozi i ugledu uvaženog profesora i pedagoga. Život vredan poštovanja! Dug radni vek prepun ostvarenja zabeleženih u njegovoj biografiji i bibliografiji.

Šta ostaje, posle profesora? Sigurno, velika praznika i tuga u životima njegovih najbližih. Ali, ono što nije zapisano i nije zabeleženo ni u jednoj službenoj evidenciji i bibliografiji je činjenica da je profesor bio zaista izuzetno odan svojoj porodici, brižan roditelj i otac, veran životni saputnik svojoj supruzi Mirjani, profesoru Farmaceutskog fakulteta, koju je nadživeo punih deset godina. Ono što je posebno krasilo njegov karakter je predusretljivost, neposrednost i humanost kojom je privlačio i toplina u izgovorenim rečima koje je birao za svakoga i u svakoj prilici. Ovakav stav je odražavao samopouzdanje i roditeljsku brigu koju je on nesebično darivao svojim saradnicima. Svako od nas mu se mogao obratiti i dobiti pomoć, savet ili sugestiju ne samo u stručnom smislu, na profesionalnom planu, već i na ljudskom, ličnom planu.

I na kraju, dodala bih i to da su njegova marljivost, savesnost i ljubav prema farmaciji i poslu kojim se bavio, a pre svega ljubav prema istoriji farmacije, pozitivno uticali na mene. On je uvek bio spreman da pomogne i od njega se moglo mnogo naučiti. Predavanja su mu bila uzorna i inspirativna, bilo da je predavao studentima ili kolegama u praksi. Plenio je govorničkim darom i punoćom izražavanja, što je često fasciniralo auditorijum. Biti u njegovom prisustvu, razgovarati i sarađivati sa njim - značilo je steći veliko profesionalno iskustvo, privrženost iskrenog prijatelja i ujedno uživati u velikoj privilegiji koja nikoga od nas nije zaobišla, a naročito mene kao njegovog bliskog saradnika.

Neka mu je večna slava i hvala!

prof. dr Dušica Krajnović
Farmaceutski fakultet Univerziteta u Beogradu

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

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