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World Pneumonia Day is observed on November 12 worldwide every year, to promote interventions in protecting against pneumonia, as well as in preventing and treating the disease. The day was marked for the first time in 2009 by the Global Coalition against Childhood Pneumonia and since then it has been a symbol of joint opposition to this disease around the world.

Pneumonia affects people in all age groups but it is particularly life-threatening for kids and persons above age of 65 years. Although the disease is easily preventable and treatable, the number of deaths by the disease is huge. This year the World Pneumonia Day is marked amid the global COVID-19 pandemic that is dramatically increases mortality from pneumonia and other causes. According to the Stop Pneumonia Initiative, COVID-19 could increase all-cause pneumonia deaths by more than 75%.

The Editorial Board of the „Vojnosanitetski Pregled" calls on all its associates to make maximum efforts to combat the pandemic and reduce the risk of pneumonia.

Svetski dan upale pluća obeležava se 12. novembra širom sveta svake godine kako bi se promovisale intervencije u zaštiti od ove bolesti, kao i njenoj prevenciji i lečenju. Taj dan je 2009. godine prvi put obeležen od strane Globalne koalicije protiv dečije pneumonije i od tada predstavlja simbol zajedničkog suprostavljanja ovoj bolesti širom sveta.

Upala pluća utiče na ljude u svim starosnim grupama, ali je posebno opasna po život dece i osoba starijih od 65 godina. Iako se bolest može lako može sprečiti i izlečiti, broj smrtnih slučajeva od nje je ogroman. Ove godine Svetski dan upale pluća obeležava se usred globalne pandemije COVID-19 koja dramatično povećava smrtnost od upale pluća. Prema Inicijativi za zaustavljanje upale pluća, COVID-19 mogla bi povećati smrtnost od svih uzroka upale pluća za više od 75%.

Uređivački odbor časopisa „Vojnosanitetski pregled" poziva sve svoje saradnike da ulože maksimalne napore u suzbijanju ove pandemije i smanjenju rizika od upale pluća.

ORIGINAL ARTICLES

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Montgomery–Asberg depression rating scale in clinical practice: Psychometric properties on Serbian patients

Montgomeri–Ašbergova skala za procenu depresivnosti u kliničkoj praksi: psihometrijska svojstva na bolesnicima u Srbiji

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Abstract

Background/Aim. Various rating scales for depression are avalable, but the Montgomery-Asberg Depression Rating Scale (MADRS) is one of the most frequently used scales. The aim of this study was to analyze the measurement properties of the MADRS Serbian version for quantifying depression severity in the clinical setting. **Methods**. Two studies have been conducted in order to validate the MADRS. The first study included sixty-four adult patients with major depressive disorder (MDD), with test-retest situation, and the second one included 19 participants (also with MDD), who had six test-retest situations. Psychometric evaluation included descriptive analysis, internal consistency and test-retest reliability, and concurrent validity (correlations with the Hamilton Depression Rating Scale 17 – HAMD-17). **Results.** The internal consistency for test-retest reliability was 0.93 in total for the MADRS, and for six test-retest situations was 0.95. The MADRS had one factor structure, with explained variance of 66.26% for the first testing, and 61.29% for the retest. There were statistical significant correlations between the MADRS and HAMD-17 ($r = 0.96$ for test and $r = 0.94$ for retest). Also, it was shown a great correlation between all items on the MADRS, and for the instrument in total ($r = 0.89$). **Conclusion**. The MADRS was shown good statistical results, and it could be used in everyday clinical practice for discriminating MDD.

Key words:
depression; sensitivity and specificity; severity of illness index; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Razne skale za procenu depresije su dostupne, ali je Montgomeri-Ašbergova skala za procenu depresivnosti (MADRS) jedna od najviše korišćenih. Cilj istraživanja bio je da se analiziraju merne karakteristike srpske verzije MADRS za procenu ozbiljnosti depresije u kliničkim uslovima. **Metode.** Sprovedena su dva istraživanja kako bi se validirala MADRS. Prva studija obuhvatila je 64 odraslih bolesnika sa velikim depresivnim poremećajem (MDD), sa test-retest situacijom, a druga je obuhvatila 19 učesnika (takođe sa MDD), koji su imali šest test-retest situacija. Psihometrijska procena se bazirala na deskriptivnoj analizi, unutrašnjoj konzistenciji i test-retest pouzdanosti, kao i konkurentnoj validnosti (korelacije sa Hamiltonovom skalom za procenu depresivnosti 17 – HAMD-17). **Rezultati.** Interna konzistentnost za test-retest pouzdanost iznosila je 0,93 za ceo MADRS instrument, dok je za šest test-retest situacija iznosila 0,95. MADRS je pokazala jednofaktorsku strukturu, koja objašnjava 66,26% varijanse za prvo testiranje, odnosno 61,29% za retest. Utvrđena je statistički značajna korelacija između MADRS i HAMD-17 ($r = 0,96$ za test i $r = 0,94$ za retest). Takođe, utvrđena je značajna korelacija između svih stavki na MADRS pojedinačno, ali i za ceo instrument ($r = 0,89$). **Zaključak**. Skala MADRS pokazala je dobre statističke rezultate i mogla bi se koristiti u svakodnevnoj kliničkoj praksi za diskriminaciju MDD.

Ključne reči:
depresija; osetiljvost i specifičnost; bolest, indeks težine; ankete i upitnici.

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Introduction

The diagnostic code for major depressive disorder (MDD) is based on episodic course, current severity, presence of psychotic features, and remission status [1]. Quantifying MDD severity and defining remission in research and clinical settings is mainly based on symptom rating scales, which are self-ratings or administered by clinicians. Various rating scales for depression are available [2], but the Montgomery-Asberg Depression Rating Scale (MADRS) is one of the most frequently used scales to quantify severity in clinical trials and everyday clinical practice [3].

Accumulated evidence from studies with different groups of people with depressive disorders indicates that the MADRS has sound psychometric properties in terms of good internal consistency, test-retest stability, and convergent validity [4–7]. It was also shown that the MADRS total score has sound construct validity for an unidimensional measure targeting core depressive symptoms [4, 5], and it provides the most accurate reflection of depression severity in overall [7]. Some studies reported that the construct of the MADRS might be represented by two to three factors underlying different depressive symptoms, such as dysphoria, retardation, and vegetative symptoms [8, 9], which should be considered in evaluating depression treatment. Good reliability and validity were also reported for the MARDS in different language versions, such as Bangla [10], Brazilian [11], Chinese [12], French [13], Korean [14], Malay [15], Persian [16], Spanish [17, 18], and Thai [19].

Research on the compatibility of the scale between the original version of the MADRS showed that there is a moderate to high association between patient and physician results [13, 20]. Also, it was examined whether the results of the MADRS were better when it was done with or without a structured interview, and the results showed that the scale had satisfactory reliability, regardless of whether the structured interview was used or not [3]. Analyzing each item individually, the MADRS has all responsive responses and the end result is more sensitive to changes in treatment [21].

The MADRS shows greater sensitivity in distinguishing between moderate and severe depression compared to the Hamilton Depression Rating Scale (HAMD) (sensitivity 93.5%, specificity 83.3%) [22]. Also, in comparison with the HAMD, significantly higher results are obtained, and it is considered to be a calibration of the scope of both instruments, that is, that the results would be equated if the cut-off score for the MARDS depression was 12, instead of the original 6 [23]. Possible shortened versions for the HAMD and MADRS were also examined without items related to somatic symptoms (e.g. sleep, appetite, etc.) [24]. In case that only a rough screen is needed, shorts version of the instruments can be used, but if the scales are used for diagnostic purposes, then it is recommended to have a full version of both scales.

The translation into Serbian for the MADRS instrument was previonsly done twice in 2008 and in 2012.

The MADRS in Serbian language has not yet been standardized. The aim of this study was to analyze psychometric properties of the MADRS Serbian version in the clinical settings.

Methods

Study 1

Questionnaires

The first scale we used was the MADRS [3]. The MADRS is the clinician-rated 10-item scale with the following items: 1) apparent sadness; 2) reported sadness; 3) inner tension; 4) reduced sleep; 5) reduced appetite; 6) concentration difficulties; 7) lassitude; 8) inability to feel; 9) pessimistic thoughts; and 10) suicidal thoughts. Answers to all items are given on the 7-point Likert scale ranging from 0 = not at all to 6 = definitively, with higher scores reflecting more severe depression symptoms. The total score is the sum of all answered items.

The second scale that was used was the HAMD, version with 17 items (HAMD-17 [25, 26]). The HAMD is the clinician-rated scale with the following items and response options: 1) depressed mood 0–4; 2) feelings of guilt 0–4; 3) suicide 0–4; 4) early insomnia 0–2; 5) middle insomnia 0–2; 6) late insomnia 0–2; (7) work and activities 0–4; 8) retardation 0–4; 9) agitation 0–4; 10) psychic anxiety 0–4; 11) somatic anxiety 0–4; 12) gastrointestinal somatic symptoms/appetite 0–2; 13) general somatic symptoms 0–2; 14) genital symptoms 0–2; 15) hypochondriasis 0–4; 16) loss of weight 0–2; and 17) insight 0–2. These symptoms are rated to cover the 1-week period prior to the interview. The total score is the sum of all answered items, with higher scores reflecting more severe depression. The HAMD had the internal consistency reliability of 0.90 in the present study.

Participants

All adults aged 18 year and above, admitted to daily hospital between June and September 2017, were eligible. The main inclusion criterion was the diagnosis of a unipolar MDD episode. Exclusion criteria were the presence of any other psychiatric and/or neurological disorder or a major somatic problem (e.g. chronic illness, impairment). All patients were diagnosed according to the International Classification of Diseases, 10th revision (ICD-10) [27] and to all was initiated some kind of treatment; antidepressant medications, social therapy, and/or psychotherapy.

A total of 64 patients, from which 36 (56.3%) were females and 28 (43.8%) males, participated in the research. Age of subjects varied from 24 to 68 years with mean of 46.11 [standard deviation (SD) = 10.85] years. The subject who were included in the study were only those who provided all the data, and only they were considered in each shown analysis.

Assessment

The MADRS was administered to all subjects independently by the first author. The same rater administered the HAMD-17. The MADRS and HAMD-17 were administered again to all subjects by the same rater two weeks later (test-retest assessment). Only subjects who appeared on the scheduled assessment after four weeks were assessed with the MARDS.

Psychometric analysis

The reliability assessment of the MADRS included internal consistency tested by the intraclass correlation coefficient (ICC), the two-way random method of absolute agreement [28]. Concurrent validity was assessed using the Pearson's correlation coefficient, and paired sample *t*-test for comparing between item results.

Study 2

Only the MADRS instrument, which characteristics were described previously, was used in the study 2. The administration of the instrument was done by the fist author, and unlike the study 1, where only one test and retest had been done, in this study six tests were done.

Participants

A total of 19 subjects participated from which 9 (47.4%) were females and 10 (52.6%) males. There was one dropout from the study, because the patient (female) was not shown to control after fourth administration. The age of the participants varied from 28 to 63 (mean = 47.32, SD = 11.06) years. Participants in this study had been also included in the study 1, but in that study were only subjected to the first two tests.

Psychometric analysis

The similar assessments were done like in the study 1: test-retest reliability by the interclass correlation coefficient, the Pearson's correlation for concurrent validity, and *t*-test for six testing situations.

Results

Study 1

Differences in impacts between items on the HAMD-17, and those on the MADRS were estimated with paired sample *t*-test. It was shown that there was statistically significant difference between several items (Tables 1 and 2).

Statistically significant results for both test and retest situations were observed in items listed in Table 2. According to the Cohen's *d*, we found that the HAMD-17 items 3, 6, 7, 10, 11, and 13 had small impact, item 9 had no significant impact, and only item 8 had moderate impact. In case of the MADRS, the Cohen's *d* showed that items 1, 6, 7, 8, and 10 had significant, but small impact, and the item 3 had no impact. For both instruments sums were statistically significant, and *d* had small effect size ($d = 0.31$ for the MADRS, and $d = 0.32$ for the HAMD-17).

The ICC for test-retest reliability was 0.93 in total [95% confidence interval (CI) 0.88–0.96; $p < 0.001$] for the

Table 1

The HAMD-17 test and retest results using *t*-test, ICC and reliability for each item

Number of item	Test	Retest	*t*	Cohen's d	*r*	ICC	95% CI	α	
	mean ± SD	mean ± SD						T1	T2
1	1.36 ± 1.44	1.11 ± 1.24	1.98	0.19	0.09	0.84*	0.73-0.90	0.90	0.89
2	0.86 ± 0.94	0.70 ± 0.85	1.86	0.18	0.09	0.84*	0.73-0.90	0.90	0.90
3	0.33 ± 0.69	0.16 ± 0.44	3.01*	0.29	0.15	0.82*	0.70-0.89	0.91	0.90
4	0.58 ± 0.75	0.48 ± 0.59	1.76	0.15	0.07	0.89*	0.82-0.93	0.90	0.89
5	0.50 ± 0.69	0.44 ± 1.31	0.36	0.06	0.03	0.21	-0.30.-0.52	0.91	0.90
6	0.55 ± 0.75	0.38 ± 0.58	3.01*	0.25	0.13	0.87*	0.79-0.92	0.91	0.89
7	1.65 ± 1.27	1.25 ± 1.19	3.40*	0.33	0.16	0.84*	0.74-0.90	0.90	0.89
8	0.98 ± 0.77	0.61 ± 0.68	5.20*	0.51	0.25	0.81*	0.69-0.89	0.91	0.89
9	0.86 ± 1.17	0.67 ± 0.99	2.55**	0.18	0.09	0.92*	0.87-0.95	0.91	0.89
10	1.05 ± 1.02	0.73 ± 0.84	4.07*	0.34	0.17	0.88*	0.80-0.93	0.91	0.89
11	0.80 ± 1.04	0.53 ± 0.85	3.28*	0.28	0.14	0.87*	0.78-0.92	0.91	0.89
12	0.30 ± 0.53	0.28 ± 0.52	0.30	0.04	0.02	0.81*	0.69-0.88	0.91	0.90
13	0.77 ± 0.81	0.61 ± 0.77	2.01**	0.20	0.10	0.82*	0.70-0.89	0.92	0.90
14	0.48 ± 0.64	0.41 ± 0.58	1.69	0.11	0.06	0.90*	0.84-0.94	0.91	0.90
15	0.31 ± 0.66	0.28 ± 0.65	0.47	0.05	0.02	0.80*	0.68-0.88	0.91	0.90
16	0.19 ± 0.47	0.16 ± 0.48	0.62	0.06	0.03	0.79*	0.65-0.87	0.91	0.90
17	0.09 ± 0.39	0.03 ± 0.18	1.43	0.20	0.10	0.48**	0.15-0.69	0.91	0.90
HAMD-17 total	11.71 ± 9.66	8.83 ± 8.50	4.75*	0.32	0.16	0.92*	0.88-0.95	0.94	0.95

***Correlation is significant at p < 0.01; **Correlation is significant at p < 0.05.**
HAMD – Hamilton Depression Rating Scale; ICC – intraclass correlation coefficient;
SD – standard deviaton; CI – confident interval.

Table 2

The MADRS test and retest results using *t*-test and ICC for each item

Number of item	Test	Retest	*t*	Cohen's d	r	ICC	95%CI	α	
	mean ± SD	mean ± SD						T1	T2
1	2.63 ± 1.83	1.94 ± 1.73	3.83*	0.39	0.19	0.81*	0.68-0.88	0.94	0.92
2	2.05 ± 1.89	1.69 ± 1.82	1.81	0.19	0.10	0.78*	0.63-0.86	0.93	0.91
3	1.80 ± 1.57	1.55 ± 1.44	2.29**	0.17	0.08	0.91*	0.85-0.94	0.94	0.92
4	1.61 ± 1.89	1.41 ± 1.67	1.85	0.11	0.06	0.94*	0.89-0.96	0.93	0.91
5	1.11 ± 1.72	0.88 ± 1.32	1.49	0.15	0.08	0.80*	0.67-0.88	0.94	0.92
6	1.98 ± 1.84	1.45 ± 1.60	3.82*	0.31	0.15	0.88*	0.81-0.93	0.93	0.91
7	2.27 ± 1.71	1.69 ± 1.58	3.84*	0.35	0.17	0.85*	0.75-0.91	0.93	0.91
8	1.88 ± 1.78	1.45 ± 1.55	2.68*	0.26	0.13	0.84*	0.73-0.90	0.93	0.91
9	1.39 ± 1.39	1.11 ± 1.20	1.99	0.22	0.11	0.76*	0.61-0.86	0.94	0.92
10	0.70 ± 1.14	0.30 ± 0.63	4.46*	0.43	0.21	0.81*	0.69-0.89	0.94	0.92
MADRS-tot	17.41 ± 13.67	13.45 ± 11.44	4.82*	0.31	0.16	0.93*	0.88-0.96	0.95	0.94

***Correlation is significant at p < 0.01; **Correlation is significant at p < 0.05. MADRS – Montgomery-Asberg Depression Rating Scale; ICC – intraclass correlation coefficient; CI – confident interval.**

MADRS, and 0.92 for the HAMD-17 in total (95% CI 0.88-0.95; $p < 0.001$). As for each item, all items on the the MADRS had significant and large impact (ICC = 0.76–0.94), and the HAMD-17 had similar results. Exception was the item 5, about transitory insomnia, where was no statistical significance. All other items had ICC values that were high and significant (ICC = 0.81–0.92). These results showed that both instruments were stable through time, and they could show changes in a patient's reaction in treatment of depression.

All items on the HAMD-17 showed significant reliability, with α = 0.89 or higher, and α = 0.91 or higher for the MADRS. According to George and Mallery [29], all α values above 0.7 are acceptable, 0.8 are good, and 0.9 are excellent. Following that rule, in this research it was shown that the MADRS had better reliability coefficients for each item than the HAMD-17, but the total scores showed similar reliability that was considered excellent (the HAMD-17: α = 0.94 for test, α = 0.95 for retest; the MADRS: α = 0.95 for test, and α = 0.94 for retest).

Table 3

Pearson's correlation for the HAMD-17 and MADRS for test and retest situations

HAMD-17 items		MADRS items	
df = (N-2)	0.01	df = (N-2)	0.01
1	0.726*	1	0.676*
2	0.723*	2	0.634*
3	0.761*	3	0.834*
4	0.825*	4	0.886*
5	0.141	5	0.687*
6	0.613*	6	0.800*
7	0.720*	7	0.735*
8	0.688*	8	0.723*
9	0.864*	9	0.624*
10	0.797*	10	0.807*
11	0.784*		
12	0.680*		
13	0.691*		
14	0.822*		
15	0.672*		
16	0.648*		
17	0.424*		
Sum(17)	0.866*	Sum(10)	0.878*

***Correlation is significant at p < 0.001 level; HAMD – Hamilton Depression Rating Scale; MADRS – Montgimery-Asberg Depression Rating Scale.**

The correlation analysis showed that there were high correlations between items on test and retest (Table 3). The MADRS had significant correlations for each item on test and retest, and coefficinet of correlation (*r*) varied from 0.62 to 0.89 ($p < 0.001$). The sum results also showed high correlation ($r = 0.89$, $p < 0.001$). Similar correlations were found also for the HAMD-17, with correlations between items on test and retest demonstrating significant correlations for all items except one (the item 5 for test and retest showed nonsignificant correlations). The coefficients of correlations varied from 0.42 to 0.86 ($p < 0.001$); for the sum, correlations were also significant ($r = 0.87$, $p < 0.001$). There were statistically significant correlations between the MADRS and HAMD-17. For the first testing, coefficient of correlation was $r = 0.96$ ($p < 0.001$), and for the retest, it was $r = 0.94$ ($p < 0.001$).

Factor analysis showed that it could be extracted one factor for both test and retest items (Table 4). For the test situation, it was shown that one factor explains 66.26% ofthe variance, and for the retest, it was explained by 61.29% of the variance. These results were as it was hypothesized, because it was supposed to be extracted one factor for the MADRS, supposing that it was measuring one factor – depression.

Study 2

The ICC for test-retest reliability was 0.95 in total (95% CI 0.90–0.98; $p < 0.001$), as it was shown in Table 5. All the items for six test-retest situations showed significance at the level $p < 0.001$, and the ICC varied from 0.77 to 0.95. This showed that with six tests, the MADRS still had good stability throughout time, at least for a period of one and a half month of the treatment in clinical conditions.

As for the reliability analysis, all six test had α = 0.91 or higher for each item, as it was the case for the sum results implying an excellent reliability by each item and in total for the MADRS (Table 5).

Table 4

Factor loadings and communalities for the MADRS based on a principal components analysis for 10 items, for both test and retest situations

Number of item	Test		Retest	
	factor loading	variance explained	factor loading	variance explained
	1		1	
1	0.731	0.534	0.772	0.596
2	0.878	0.771	0.814	0.662
3	0.810	0.656	0.747	0.558
4	0.858	0.735	0.854	0.730
5	0.751	0.564	0.617	0.381
6	0.907	0.822	0.837	0.701
7	0.817	0.667	0.839	0.705
8	0.849	0.721	0.868	0.753
9	0.738	0.545	0.668	0.446
10	0.781	0.610	0.773	0.597

***Note*: Every loading greater than 0.30 is considered significant.**
MADRS – Montgomery-Asberg Depression Rating Scale.

Table 5

The MADRS for six test-retest results using ICC and reliability for each item

Number of item	ICC	95% CI	α					
			T1	T2	T3	T4	T5	T6
1	0.93*	0.87-0.97	0.94	0.94	0.93	0.94	0.93	0.91
2	0.93*	0.87-0.97	0.94	0.94	0.93	0.94	0.93	0.92
3	0.77*	0.56-0.90	0.95	0.95	0.95	0.95	0.94	0.93
4	0.88*	0.77-0.95	0.95	0.95	0.94	0.95	0.94	0.94
5	0.92*	0.85-0.97	0.95	0.95	0.96	0.95	0.94	0.93
6	0.95*	0.90-0.98	0.95	0.94	0.94	0.94	0.93	0.92
7	0.95*	0.90-0.98	0.95	0.94	0.94	0.94	0.93	0.91
8	0.94*	0.88-0.97	0.94	0.94	0.93	0.94	0.93	0.92
9	0.93*	0.87-0.97	0.95	0.94	0.94	0.94	0.93	0.92
10	0.90*	0.81-0.96	0.96	0.95	0.95	0.95	0.94	0.93
MADRS-tot	0.95*	0.90-0.98	0.94	0.93	0.93	0.93	0.94	0.95

***Correlation is significant at p < 0.001.**
MADRS – Montgomery-Asberg Depression Rating Scale;
ICC – intraclass correlation coefficient; CI – confidence interval.

The correlation results showed that there were high correlations (Table 6). The MADRS had significant correlations for all six retests, and the coefficients of correlations varied from 0.51 to 0.98, with significance at $p < 0.01$ or $p < 0.05$. Higher correlations were shown for tests that had a closer time interval, unlike those that had more distant time interval. Also, higher correlations at the significance level $p < 0.01$ were shown in the first testing, and for the sixth retest showed smaller correlation at $p < 0.05$.

Discussion

The multivariable analysis showed that the MADRS possesses appropriate reliability and concurrent validity. The internal consistency reliability of the MADRS in Serbian language was high as well as corrected item-total correlations, what pictures high homogeneity among the items in measuring the intended concept and the consistency in rating the severity across the items even when considering individual assessments [28, 29]. The ICC for the study 1 was 0.93 in total (95% CI 0.88–0.96; $p < 0.001$) for the MADRS, and 0.95 in total for the study 2 (95% CI 0.90–0.98; $p < 0.001$). High internal consistency reliability for the MADRS total score, with Cronbach's alpha coefficient above 0.8, was previously observed across studies using the original and different language versions [5, 7, 16, 19]. In addition, the test-retest reliability of the MADRS in Serbian was excellent, for both studies 1 and 2, whereas in both α was 0.91 and higher, indicating satisfactory stability in repeated measurements.

The factor analysis showed that one factor explained most of the variance (66.26% of the variance for the first testing, and for the retest it was explained by 61.29% of the variance), as it was expected. Other studies have found more factors that could explain variance, that is, three [30], or two [31], depending on the study. This may be due to smaller sample size in our study, and these results should be confirmed in later research.

Finally, concurrent validity reported previously [11, 15, 16] was also evident for the MADRS total score for the Serbian version when tested against the HAMD-17 total score. The correlation of MADRS with HAMD was high and significant ($r = 0.96$; $p < 0.001$ for test, and $r = 0.94$; $p < 0.001$ for the retest). Other studies have shown smaller correlations, $r = 0.58$ [32]. Higher correlations in our research might be because of the smaller sample size, so the results might be different in the future research with bigger sample. There were also significant correlations between items on the MADRS (both for test and retest; $r = 0.62–0.89$, $p < 0.001$), and on the HAMD-17 (test and retest; $r = 0.42–0.86$, $p < 0.001$). Correlations between six tests in the study 2 also were significant ($r = 0.51–0.98$, mostly $p < 0.01$). Significant correlations were also between the MADRS and HAMD-17 ($r = 0.96$; $p < 0.001$ for the first test, and $r = 0.94$; $p < 0.001$ for the retest). These results have been confirmed in other studies, where the correlations exist between items, and between the MADRS and HAMD-17 [31, 33].

Table 6

The Pearson's correlation for the MADRS for six test-retest situations

Number of retest	1	2	3	4	5	6
1	-					
2	0.979*	-				
3	0.865*	0.889*	-			
4	0.703*	0.741*	0.899*	-		
5	0.581*	0.638*	0.775*	0.918*	-	
6	0.511**	0.553**	0.657*	0.812*	0.942*	-

***Correlation is significant at p < 0.01;**
****Correlation is significant at p < 0.05.**
MADRS – Montgomery-Asberg Depression Rating Scale.

Limitations

There are several limitations of the study. First, a small number of participants did not allow to study changes in mental health in those who deteriorated during the study period. Also, the samples in both studies were small and this limited the generalizability of the studies to other settings. Further research should include a bigger sample and also comparison to a general population, for the purpose of better validity testing. The bigger sample is referred to both studies, 1 and 2.

Conclusion

In summary, this study of the MADRS in Serbian demonstrated that it is appropriate measure for routine, clinical assessments of individuals with MDD. It showed that the measure could produce reliable and valid assessments of MDD severity with possibility to distinguish a clinically important improvement from measurement error with a large amount of certainty. However, with awareness of the limitations of the present study, additional investigations will be needed with different samples in order to set the MADRS as a gold standard in routine psychiatric practice.

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Comparison of Custodiol® and modified St.Thomas cardioplegia for myocardial protection in coronary artery bypass grafting

Poređenje Custodiol®-a i modifikovane St. Thomas kardioplegije u zaštiti miokarda tokom operacije aortokoronarnog premošćavanja

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Abstract

Background/Aim. Custodiol® is a hyperpolarizing cardioplegic solution which has been used in our national cardiac surgical practice exclusively for the heart transplant surgery. Owing to its numerous advantages over the standard depolarizing solutions, Custodiol® became cardioplegic solution of choice for all other cardiac surgical procedures in many cardio-surgical centers. This study evaluated myocardial protection by Custodiol® compared to modified St. Thomas cardioplegic solution in coronary artery bypass surgery. **Methods.** In a prospective four-month study, 110 consecutive adult patients who underwent primary isolated elective on-pump coronary artery bypass grafting (CABG) were randomized into the Custodiol® group (n = 54) and the St. Thomas groupa (n = 50), based on the type of administered cardioplegia; six patients were excluded. Cardiac protection was achieved as antegrade cold crystalloid cardioplegia by one of the solutions. Myocardial preservation was assessed through following outcomes: spontaneous rhythm restoration post cross-clamp, and postpoperative cardiac specific enzymes level, ejection fraction (EF) change, inotropic support, myocardial infarction (MI), atrial fibrillation (AF), and death. **Results.** Preoperative and intraoperative characteristics of patients in both groups were similar except for a considerably longer cross-clamp time in the Custodiol® group (49.1 ± 19.0 vs. 41.0 ± 12.9 minutes; $p = 0.022$). The Custodiol® group exhibited a higher rate of return to spontaneous rhythm compared to the St. Thomas group (31.5% vs. 20.0%, respectively; $p = 0.267$), lower rates of AF (20.4% vs. 28%, respectively; $p = 0.496$), MI (1.8% vs. 10.0%, respectively; $p = 0.075$) and inotropic support (9.0% vs. 12.0%, respectively; $p = 0.651$), albeit not statistically significant. There was an insignificant difference in peak value of troponin I between the Custodiol® and Thee St. Thomas group (5.0 ± 3.92 μg/L vs. 4.5 ± 3.39 μg/L, respectively; $p = 0.755$) and creatine kinase-MB (26.9 ± 15.4 μg/L vs. 28.5 ± 24.2 μg/L, respectively; $p = 0.646$) 6 hours post-surgery. EF reduction was comparable (0.81% vs. 1.26%; $p = 0.891$). There were no deaths in both groups. **Conclusions.** Custodiol® and modified St.Thomas cardioplegic solution have comparable cardioprotective effects in CABG surgery. The trends of less frequent MI, AF and inotropic support, despite the longer cross-clamp time in the Custodiol® group may suggest that its benefits could be ascertained in a larger study.

Key words:
heart arrest, induced; custodiol-n solution; cardiac surgical procedures; postoperative period; arrhythmias, cardiac; heart function tests; mortality.

Apstrakt

Uvod/Cilj. Custodiol® je hiperpolarizirajući kardioplegični rastvor koji je korišćen u našoj nacionalnoj kardiohirurškoj praksi isključivo u transplantacionoj hirurgiji. Zbog svojih brojnih prednosti u odnosu na standardne depolarizirajuće rastvore, Custodiol® je, u mnogim kardiohirurškim centrima, postao kardioplegični rastvor izbora za sve kardiohirurške procedure. Cilj studije bio je procena zaštite miokarda rastvorom Custodiol® u poređenju sa modifikovanom St. Thomas kardioplegičinim rastvorom u koronarnoj hirurgiji. **Metode.** Tokom prospektivne četveromesečne studije, 110 uzastopnih odraslih bolesnika podvrgnutih primarnoj, izolovanoj, elektivnoj operaciji aortokoronarnog premošćavanja bili su randomizirani na osnovu primenjene kardioplegije u Custodiol® grupu (n = 54) i St. Thomas grupu

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(n = 50); šest bolesnika je bilo isključeno iz studije. Zaštita miokarda izvršena je primenom antegradne, hladne kristaloidne kardioplegije jednim od rastvora, a procenjena je praćenjem sledećih parametara: spontane uspostave srčanog ritma nakon deklemovanja; vrednosti kardiospecifičnih enzima; promene ejekcione frakcije (EF) 24 sata nakon operacije; učestalosti postoperativne inotropne potpore, infarkta miokarda (MI), atrijalne fibrilacije (AF) i smrti. **Rezultati.** Preoperativne i intraoperativne karakteristike bolesnika bile su slične u obe grupe, osim značajno dužeg vremena klemovanja u Custodiol® grupi (49,1 ± 19,0 min. vs. 41,0 ± 12,9 min; $p = 0,022$). Custodiol® grupa, u odnosu na St. Thomas grupu, imala je češću spontanu uspostavu srčanog ritma (31,5% vs. 20,0%, $p = 0,267$), uz nižu učestalost AF (20,4% vs. 28%, $p = 0,496$), MI (1,8% vs. 10,0%, $p = 0,075$) i inotropne podrške (9,0% vs. 12,0%, $p = 0,651$), ali bez statističke značajnosti. Maksimalne vrednosti troponina I (5,0 ± 3,92 μg/L vs. 4,5 ± 3,39 μg/L; $p = 0,755$) i kreatin kinaze-MB (26,9 ± 15,4 μg/L vs. 28,5 ± 24,2 μg/L; $p = 0,646$) bile su slične, šest sati nakon operacije. U obe došlo je do je sličnog smanjenja EF (0,81% vs. 1,26%; $p = 0,891$) bez mortaliteta unutar 30 dana nakon operacije. **Zaključak.** Custodiol ima sličan kardioprotektivni efekat u poređenju sa modifikovanim St. Thomas kardioplegičnim rastvorom kod operacije aortokoronarnog premošćavanja. Zbog dužeg vremena klemovanja aorte, uz istovremeni trend manje učestalosti MI, AF i inotropne podrške u grupi sa rastvorom Custadiol®, studija na većem broju bolesnika mogla bi otkriti prednosti Custadiol®-a u zaštiti miokarda tokom operacije aortokoronarnog premošćavanja.

Ključne reči:
srce, izazvani zastoj; custodiol-n rastvor; hirurgija, kardijalna, procedure; postoperativni priod; aritmija; srce, funkcijski testovi; mortalitet.

Introduction

Cardiac surgery is always accompanied by a certain degree of myocardial damage which is multifactorial and cumulative, but ischemia-reperfusion injury is the major factor inducing intraoperative myocardial damage [1]. Myocardial protective strategies during cardiac surgery aim to diminish ischemia-reperfusion myocardial injury that could cause myocardial infarction (MI), arrhythmias, ventricular dysfunction and low cardiac output syndrome (LCOS). Atrial fibrillation (AF), the need for inotropic support, acute renal injury, prolonged intensive care unit (ICU) stay, and death are the most common consequences of LCOS [1, 2]. There are numerous methods of myocardial protection during cardiac surgery, but cardiopulmonary bypass (CPB), hypothermia, and cardioplegic arrest remain the primary protective techniques during open heart surgery [2–4]. Cardioplegia, as a method of myocardial protection, has been in use for almost half a century. Crystalloid cardioplegic solutions could be categorized according to the electrolyte composition into two types: extracellular and intracellular type. Extracellular solutions contain higher concentration of sodium, calcium, and magnesium and produce depolarizing cardiac arrest. Intracellular solutions with a low concentration of sodium and calcium, induce cardiac arrest by hyperpolarization. St.Thomas Hospital solution and its modifications are the best known and longest used extracellular crystalloid cardioplegia [2]. Bretschneider histidine-tryptophan-ketoglutarate solution, known as Custodiol® (Custodiol® HTK, Köhler Chemie GmbH, Bensheim, Germany), is an example of an intracellular crystalloid cardioplegic solution [4]. Custodiol® is an intracellular, hyperpolarized, crystalloid solution commonly used for organ preservation in heart transplant procedure, but its use for myocardial protection in ordinary cardiac surgery has not been established entirely and remains an off-label indication in many countries. Custodiol® solution administered in a single dose provides up to 2–3 hours of myocardial protection, which is an advantage over alternative cardioplegic solutions requiring re-administration every 20–30 min. Moreover, cardiac arrest induced by hyperpolarization mimics natural resting state of the heart and minimize metabolic demand decreasing adenosine triphosphate (ATP) depletion thus improving the conditions of the heart to be reanimated at the end of the procedure.

Custodiol® contains a low sodium concentration that causes hyperpolarization and prevents edema and a high concentration of histidine with high buffering capacity, effective under hypothermic conditions, which may enhance the efficiency of anaerobic glycolysis. Furthermore, Custodiol® contains mannitol, ketoglutarate and tryptophan. Mannitol and histidine as well are free radical scavengers that decrease cellular edema. Ketoglutarate is an intermediate in the Krebs cycle and increases energy production upon reperfusion, whereas tryptophan stabilizes cell membranes [4, 5].

Custodiol® has been used in our national cardiac surgical practice exclusively as an organ preserving solution for the heart transplant surgery, with a special supply permission, since it is not yet registered at the Medicines and Medical Devices Agency of Serbia (MMDS). Standard strategy, with modified extracellular St.Thomas cardioplegic solution has been used for decades in adult cardiac surgical patients, and thus, it is registered at the MMDS. Due to its numerous advantages over the standard, depolarizing solutions, Custodiol® became the first choice solution for all other, non-transplant cardiac surgical procedures, in the most cardiac surgical centers worldwide. Our intention to start a new strategy, with routine use of intracellular Custodiol® solution for non-transplant cardiac surgical patients, came from well known advantages of this solution.

There is still no clear evidence nor consensus among cardiac surgeons and anesthesiologists as to which cardioplegia provides the best myocardial protection. The ideal composition and method of using cardioplegic solution are still open questions [4]. The aim of this study was to evaluate myocardial protection of Custodiol® compared to modified St. Thomas cardioplegia in adult coronary artery bypass surgery.

Methods

Patient population

The prospective randomized study included 110 consecutive adult cardiac patients (pts), between February and June 2018, who underwent primary isolated and elective coronary artery bypass grafting surgery (CABG), in condition of extracorporeal circulation.

The study protocol followed the Declaration of Helsinki and was approved by the Institution's Ethics Committee. Informed consent was obtained from each patient.

Inclusion criteria were**:** adult elective coronary patients with minimun two angiographic graftable target vessels (diameter > 2.0 mm with stenosis ≥ 70%), LVEF (left ventricular ejection fraction) ≥ 30%, ultrasonography verified absence of significant valvular pathology.

Exclusion criteria were older than 80 years, myocardial infarction within 30 days of the operation, reoperations, urgent/emergent patients, off-pump CABG, left main stenosis > 50%, ongoing myocardial ischemia [verified by electrocardiogram (ECG) and elevated cardiac troponin I (cTnI) and/or creatine kinase MB (CK-MB) isoenzyme], pericarditis, serum creatinine level > 200 μmol/L, coronary artery endarterectomy, and left ventricular surgical restoration.

Patients who met the inclusion criteria of the study were randomized into two groups: the group receiving standard modified St.Thomas cardioplegic solution (St. Thomas M) according to the Hospital protocol, and the group receiving one bolus (20 mL/kg) of Custodiol® HTK Solution, during six to eight minutes (Table 1).

Six patients were subsequently excluded from the study after randomization due to: myocardial infarction during anesthesia induction – 1 patient, errors in the cardioplegia delivery protocol – 3 patients, endarterectomy of coronary artery – 1 patient, and left ventricular restoration surgery – 1 patient.

Anesthesia and operative technique

Anesthetic induction was performed with midazolam (0.1 mg/kg), hypnomidate (0.2 mg/kg), rocuronium bromide (0.6 mg/kg) and sufentanil (1 μg/kg). Maintenance of anesthesia was provided by sufentanil (0.02–0.05 μg/kg/min), sevoflurane (0.8–1.5 vol%) and rocuronium bromide (8–10 μg/kg/min). Standard operative technique through total median sternotomy was used for all patients. Cardiopulmonary bypass in condition of mild systemic hypothermia (32 °C to 34 °C) was established with ascending aortic cannulation and right atrial two-stage venous cannulation after systemic heparinization (4 mg/kg) with a target activated clotting time greater than 480 s. Standard management included membrane oxygenators, roller pump with a non-pulsatile flow of 2–2.4 L/min/m^2 with a mean arterial blood pressure around 60 mmHg. After CPB was discontinued, heparin was neutralized with 0.8 mg protamine sulfate *per* 1 mg of heparin. Cell saver and tranexamic acid (30 mg/kg) were routinely used. Myocardial protection was achieved by one of the two study solution as antegrade intermittent cold crystalloid cardioplegia (4–8ºC) and topical cooling with iced saline "slush". Cardioplegic solutions were administrated as follows: St.Thomas M induction dose was 1,000 mL over 3–5 min with maintenance doses of 200 mL over 2 min every 20 min thereafter; Custodiol® was delivered as one single dose of 20 mL/kg over 6–8 min whereas the second dose was provided only when the cross-clamp time exceeded 120 min. If the heart exhibited electrical or mechanical activity during the procedure, additional doses of 200 mL of the cardioplegic solution was administered. The composition of modified St Thomas [5] cardioplegic solution routinely used in our Institution was: Na^+ 147 mM/L, K^+ 20 mM/L, Mg^{2+} 16 mM/L, Ca^{2+} 2 mM/L, Cl^- 203 mM/L, $NHCO3^-$ 10 mM/L, Osmolality 388 mOsm/kg, pH(25°C) ~ 7.8, and the composition of the Custodiol® [7] was: Na^+ 15 mM/L, K^+ 9 mM/L, Mg^{2+} 4 mM/L,Ca^{2+} 0.015 mM/L, histidine 198 mM/L, Tryptophan 2 mM/L, ketoglutarate 1 mM/L, mannitol 30 mM/L, osmolality 310 mOsm/kg, pH(25°C) ~ 7.02–7.20.

Perioperative transfusion, fluid administration, and use of inotropes and vazopressor were carried out at the discretion of anesthesiologists.

As primary outcome measures we compared: cTnI levels preoperative, 6, 24 and 48 hours post surgery, and changes in LVEF by transthoracic echocardiogram (TTE), 24 hours post surgery.

As a secondary outcome measures, we compared: 30-day mortality; CK-MB levels preoperative, 6, 24 and 48 hours post surgery; time to cardiac arrest (time elapsed from the introduction of cardioplegic infusion to the cessation of cardiac electrical and mechanical activity); spontaneous rhythm restoration post aortic cross-clamp (ACC); prolonged mechanically assisted ventilation (MV) ≥ 24 h, up to 48 hours post surgery; inotropic support ≥ 60 min, up to 48 hours post surgery; MI, up to 48 hours post surgery; AF *de novo*, up to 48 hours post surgery; prolonged ICU and hospital length of stay (LOS) > 3 days; other postoperative morbidity [infection (deep wound infection or sepsis), stroke, acute kidney injury].

Data collection and definitions

Twelve-lead ECG was routinely obtained preoperatively at the ICU at arrival and daily until ICU discharge, and whenever the clinical situation of the patient required it. All ECGs were compared with the preoperative recording for evidence of new postoperative infarction. Heart rhythm and rate were monitored continuously with telemetry during the ICU stay. Patients who suffered at least one episode of AF postoperatively, without history of AF preoperatively, and needed medical treatment were recorded.

TTE examinations were performed preoperatively and about 24 hours post-surgery by two highly experienced echocardiographers. Left ventricular (LV) ejection fraction was estimated by biplane Simpson's method. Regional wall motion abnormalities were visually assessed and were

marked as akinetic, dyskinetic, or hypokinetic segments. Occurrence of new postoperative segmental wall motion defects or deterioration of the existing one, compared with the preoperative echocardiography, were registered. Left ventricle walls thickness more than 11 mm was considered as a marker of left ventricular hypertrophy.

Cardiac markers (cTnI, CK-MB) were determined in peripheral blood preoperatively (T0), at 6 (T6), 24 (T24) and 48 (T4) hours postoperatively according to profile of enzymes release after on-pump CABG [7]. cTnI and CK-MB were measured quantitatively by means of enzyme electrochemiluminescence immunoassay (Beckman Coulter Access 2 Analyzer): the upper normal reference limit (URL, 99th percentile) for cTnI was 0.05 μg/L and for CK-MB these values were 7.2 μg/L for male and 3.4 μg/L female.

Criteria for MI were: peak of cTnI value > 3.1 μg/L within 48 hours after operation, with normal preoperative values, associated with either ECG showing new pathological Q waves or new left bundle branch block (LBBB) or TTE revealing new regional hypokinetic or akinetic area in the left or right ventricle [6, 8].

Acute kidney injury (AKI) was defined as an increase in serum creatinine to ≥ 2 times baseline

Statistical analysis was performed using the IBM SPSS statistics for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ±standard deviation or median and interquartile range, whilst categorical variables were given as absolute values and percentages.

Table 1

Baseline characteristics and patient comorbidities

Parameters	Custodiol® group (n = 54)	St Thomas M group (n = 50)	*p value*
Age (years)	64.5 ± 6.5(65.0; 61.8–69.0)	65.3 ± 6.3(65.00; 61.8–70.0)	0.546[a]
Male sex	40 (74.1)	44 (88.0)	0.121[b]
BMI (kg/m^2)	1.95 ± 0.18(1.94;1.87-2.02)	1.96 ± 0.14(1.96;1.85-2.04)	0.970[a]
Risk factors			
Hypertension	40 (74.1)	44 (88.0)	0.121[b]
Diabetes mellitus/insulin-treated	24 (44.4)/4 (7.4)	20(40.0)/11(22.0)	0.795/0.066[b]
Dyslipidemia	24 (44.4)	24 (48.0)	0.868[b]
Active smoker	21 (38.9)	21 (42.0)	0.902[b]
Comorbidity non cardiac			
CKD:GFR < 60 mL/min/1.73 m^2	8 (14.8)	9 (18.0)	0.862[b]
COPD	3 (5.6)	5 (10.0)	0.630[b]
Carotid disease	5 (9.3)	5 (10.0)	1.000[b]
PVD	5 (9.3)	4 (8.0)	1.000[b]
Comorbidity cardiac			
Previous LVEF	57.9 ± 7.9 (58.0;52.0–64.0)	55.2 ± 8.1(56.0; 48.8–62.5)	0.116[a]
LV hypertrophy	10(18.5)	3 (6.0)	0.103[b]
Atrial fibrillation	11(20.4)	6 (12.0)	0.375[b]
NYHA class	1.57 ± 0.60 (2;1–2)	1.6 ± 0.62 (2;1–2)	0.673[c]
CCS	2.09 ± 0.87 (2;2–2)	1.86 ± 0.88 (2;1–2)	0.363[c]
Prior MI *per* patient	0.76 ± 0.08 (1;0–1)	0.82 ± 0.66 (1;0–1)	0.709[c]
Prior MI n (%) of patients	38 (70.4)	34 (68.0)	0.289[b]
STEMI/NSTEMI	18 (42.9)/24 (57.1)	22 (53.7)/19 (46.3)	0.302/0.348[b]
Prior PCI	14 (26.4)	12 (24.0)	0.956[b]
Diseased vessels	3.37 ± 0.83 (3; 3–4)	3.14 ± 0.83 (3; 3–4)	0.192[c]
Syntax score	29.8 ± 9.4 (29.0; 22.0–36.5)	29.1 ± 8.8 (28.0; 21.9–35.5)	0.713[c]
EuroScore II	1.15 ± 0.73 (0.92; 0.64–1.59)	1.14 ± 0.48 (1.10 ; 0.71–1.54)	0.452[a]
Preoperative drug therapy			
β-blockers	46 (85.2)	44 (88.0)	0.894[b]
Loop diuretics	10 (18.5)	7 (14.0)	0.721[b]
Acetylsalicylic acid	54 (100.0)	49 (98.0)	0.969[b]
trimetizidine	5 (9.3)	15 (30.0)	0.015[b]
Statins	42 (77.8)	44 (88.0)	0.264[b]
ACEI/ARB	51 (94.4)	46 (92.0)	0.916[b]

***Note*: Results are given as mean ± standard deviation (median; interquartile range) or number (percentage). BMI – body mass index; COPD – chronic obstructive pulmonary disease; PVD – peripheral vascular disease; MI – myocardial infarction; STEMI/NSTEMI – ST/non-ST segment elevation MI; PCI – percutaneous coronary intervention; PVD –peripheral vascular disease;LV – left ventricular;LVEF – LV ejection fraction (Simpson's method);CKD –chronic kidney diseases; GFR – glomerular filtration rate; CCS – Canadian Cardiovascular Society grading of angina pectoris;NYHA – New York Heart Association; Diseassed vessels – coronary stenosis > 70%; Syntax score – angiographic score of coronary artery disease complexity; Euro score – European System for Cardiac Operative Risk Evaluation; ACEI – angiotensin converting enzyme inhibitor; ARB – angiotensin receptor blocker.**

[a]*t* test; [b]χ^2 test; [c]Mann-Whitney U test.

Table 2

Operative data and postoperative outcomes

Parameters	Custodiol® group (n = 54)	St Thomas M group (n = 50)	*p value*
CPB time (min)	82.2±23.7 (80.0; 63.3–94.0)	74.5 ± 18.5 (70.5; 60.0–84.0)	0.075[c]
ACC time (min)	49.1 ± 19.0 (45.5; 35.8– 60.2)	41.0 ± 12.9 (39.0; 30.0–50.2)	0.022[c]
Number of grafts	2.9 ± 0.9 (3;2–4)	2.7 ± 0.8 (3; 2–3)	0.593[c]
LITA	52 (96.3)	49 (98.0)	1.000[b]
T (sec)	59.3 ± 21.3	59.7 ± 30.0	0.388[c]
Amount of cardioplegia (mL)	1667 ± 268 (1600;1500 –1812)	1306 ± 251 (1300;1100–1350)	0.000[c]
Spontaneous rhytam after ACC	17 (31.5)	10 (20.0)	0.267[b]
Clinical outcomes			
LVEF postoperative	57.0 ± 8.5 (56.5; 50.0–65.0)	54.0 ± 8.0(55.0; 50.0–59.3)	0.073[a]
ΔEF	-0.8 ± 4.3(1.0; -2.0–4.0)	-1.16 ± 5.10(0.0;-2.3-5.0)	0,896[a]
Postoperative MI	1(1.8)	5(10.0)	0.075[b]
Atrial fibrillation *de novo*	11 (20.4)	14 (28.0)	0.496[b]
Inotropic support	5 (9.3)	6 (12.0)	0.893[b]
Reoperation for bleeding	2 (3.7)	4 (8.0)	0.604[b]
Drainage (mL)	745 ± 470 (635; 230–2400)	848 ± 414 (710; 444–1078)	0.292[c]
Transfusion (mL)	248 ± 345 (238; 0–303)	343 ± 414 (255; 0–559)	0.260[c]
Acute kidney injury	0 (0)	0 (0)	/
Stroke	0 (0)	0 (0)	/
Infection	1 (1.9)	1 (2.0)	1.000[b]
Prolonged MV > 24 h	0 (0)	0 (0)	/
ICU length of stay (days)	2.8 ±1.8 (2.0; 2.0–3.0)	3.4 ± 4.3 (2.0; 2.0–3.0)	0.395[c]
Prolonged ICU-LOS > 3 days	8 (14.8)	12 (24.0)	0.348[b]
Hospital (H) LOS (days)	7.7 ± 3.9 (7.0; 6.0–8.0)	8.0 ± 4.5 (7.0; 6.0–7.0)	0.811[c]
Prolonged H-LOS > 10 days	4 (7.4)	6 (12.0)	0.645[b]
30-day mortality	0 (0)	0 (0.0)	/

***Note*: Results are given as mean ± standard deviation (median; interquartile range) or number (percentage).**

CBP – cardiopulmonary bypass; ACC – aortic cross-clamp; LITA – left internal thoracic artery; T – time to cardiac arrest after ACC; LVEF – left ventricular ejection fraction; ΔEF = LVEF preoperative-LVEF 24 h- postoperative; MI – myocardial infarction; Drainage/Transfusion (mL) 48 hours post surgery; ICU – intensive care unit; LOS – length of stay; MV – mechanical ventilation; Infection – deep sternal wound infection or sepsis.

[a]*t* test; [b]χ^2 test; [c]Mann-Whitney U test.

A comparison of the two groups, i.e. two clinical treatments, was done using *t*-test and Mann-Whitney U-test for continuous variables and χ^2 test for categorical variables. *P* values < 0.0^5 were considered statistically significant.

Results

The study enrolled 104 patients of which 54 patients used Custodiol® and 50 patients used St. Thomas M cardioplegia for myocardial protection. Patients in both groups were well balanced with regard to the preoperative demographic and clinical characteristics (Table 1). Demographics data were similar, two-thirds of them were male, with a mean patient age about 65 years. There were no statistically significant differences between the subjects in any of the following variables displayed in Table 1: risk factors, non-cardiac and cardiac comorbidities. In terms of ischemic heart diseases, majority of patients had a three vessel coronary artery disease with medium to high degree of coronary disease extensity with regard to average value of SYNTAX score of 29. Furthermore, about half of patients suffered from moderate anginal disorders, class 2 according to the Canadian Cardiovascular Society (CCS) Angina Grading Scale. Most of the patients had previously survived one MI with preserved LV function and LVEF greater than 55%. They were categorized to the 2nd stage of heart failure according to the New York Heart Association (NYHA) classification. Left ventricular hypertrophy was three times more common in the Custodiol® group than in the St. Thomas M group (18.5% vs. 6.0%, respectively), but this difference did not reach statistical significance ($p = 0.103$). We noted that hypertrophied myocardium was more vulnerable to ischemic damage [1, 6]. Patients in both groups were consider to be at low surgical risk on the basis of log Euro Score II (European System for Cardiac Operative Risk Evaluation) with mean value of around 1. Considering preoperative therapy, patients in the St. Thomas M group notably more often used trimetizidine (a cardioprotective agent that reduce ischemia-reperfusion injury of the heart) compared with patients in the Custodiol® group, 30.0% vs. 9.3%, respectively ($p = 0.015$).

Table 2 presents the operative data and postoperative outcomes across the two groups. With regarding to the operative data, the average cardiopulmonary bypass time was

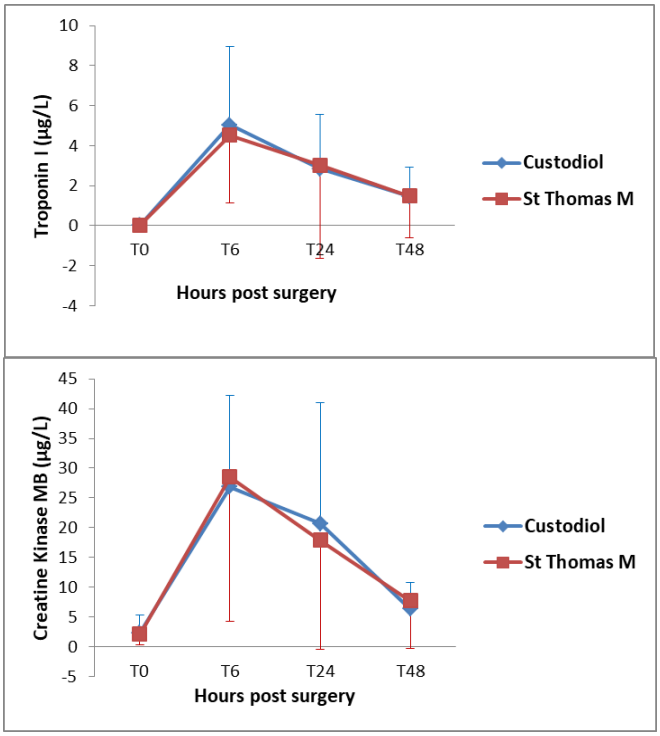

Fig. 1 – Enzyme level values over 48 hours post surgery (mean ± standard deviation).

Table 3

Cardiac enzymes at 6 and 24 hours post-surgery

Cardioplegic solution	cTnI (µg/L) peak level	CK-MB (µg/L) 6 horus post-surgery	cTnI (µg/L) prognostic level	CK-MB (µg/L) 24 hours post-surgery
Custodiol®	5.04 ± 3.92 (4.02;1.98–7.18)	26.89 ± 15.4 (25.10;14.40–37.35)	2.84 ± 2.74 (1.91; 0.91–4.13)	20.73 ± 20.20 (16.80; 8.62–23.75)
St.Thomas M	4.53 ± 3.39 (3.57;2.21–5.71)	28.50 ± 24.20 (21.30;14.42–35.38)	3.13 ± 4.68 (1.77;0.88–3.09)	18.10 ± 18.30 (12.00; 7,78–21,48)
*p**	0.755	0.646	0.571	0.164

***Note:* Results are given as mean ± standard deviation (median; interquartile range).**
cTnI – cardiac troponin I; CK-MB – creatine kinase-MB.
***Mann-Whitney U test.**

longer for the Custodiol® group compared to the St. Thomas M cardioplegia group, 82.2 ± 23.7 vs. 74.5 ± 18.5 min, respectively. This difference trended toward statistical significance with a *p* value of 0.075. The cross-clamp time difference between the groups reached statistical significance with a *p* value of 0.022 and were considerably longer for patients in the Custodiol® group (49.1 ± 19.0 min) compared to that in the the St. Thomas M group (41.0 ± 12.9 min). On the other hand, the number of grafts was similar, about 3 grafts *per* patient, and the internal mammary artery was used in over 95% of cases in both groups. Intraoperative parameters of cardioplegic efficiency, time to cardiac arrest, and spontaneous rhythm recovery rate were similar in both groups. The total amount of used cardiplegic solution was significantly higher in the Custodiol® vs. St.Thomas M group, 1,667 vs. 1,306 mL, respectively ($p = 0.000$). Postoperative complications data presented in Table 2 were comparable between the groups. With regard to postoperative clinical indicators of myocardial protection efficacy, the results were as follows: there were neither 30-day deaths nor prolonged MV over 24 hours in either group, the Custodiol® group showed less frequently AF (20.4% vs. 28.0%; $p = 0.496$), MI (1.8% vs. 10.0%; $p = 0.075$) and inotropic support (9.0% vs. 12.0%; $p = 0.651$) than the St.Thomas M group, but without statistical significance, LVEF reduction was comparable (0.81% vs. 1.26%; $p = 0.891$), prolonged ICU and hospital LOS were less frequent in the Custodiol® than in the St.Thomas M group, but with no statistical significance, (14.8% vs. 24.0%, $p = 0.348$ and 7.4% vs. 12.0% , $p = 0.645$, respectively).

In terms of cardiac enzymes, as markers of myocardial necrosis, no statistically significant difference was found in any of the sampling times as shown in Figure 1 and Table 3. The peak of the values of all enzymes was 6 after the surgery.

Composite indicator, myocardial protection efficacy (PMPE) was introduced to capture occurrence of infrequent clinically important outcomes. In particular, PMPE was defined as occurrence of one of following: postoperative MI, AF *de novo*, inotropic support, prolonged IUC lenght (ICU-LOS) > 3days, prolonged hospital LOS > 10 days, prolonged MV > 24 h, 30-day mortality, cTnI > 8.5 µg/L at 24 h post-

Table 4

Summarized factors of perioperative myocardial injury

Variable	Custodiol group (n = 54)	St Thomas M group (n = 50)	*p*
Predictors of perioperative myocardial injury and postoperative cardiac troponin I (cTnI) elevation [6, 7]			
Age (years)	64.5 ± 6.5 (65.0;61.8- 0)	65.3 ± 6.3 (65.00; 61.8-70.0)	0.546[a]
Female sex	14 (25.9)	6 (12.0)	0.121[b]
Previous LVEF	57.9 ± 7.9 (58.0; 52.0–64.0)	55.2 ± 8.1 (56.0; 48.8–62.5)	0.100[a]
LV hypertrophy	10 (18.5)	3 (6.0)	0.103[b]
Diseassed vessels	3.37 ± 0.83 (3; 3–4)	3.14 ± 0.83 (3; 3–4)	0.192[c]
CKD:GFR < 60mL/min/1.73m^2	8 (14.8)	9 (18.0)	0.862[b]
Preductal	5 (9.3)	15 (30.0)	0.015[b]
EuroScore II	1.15 ± 0.73(0.92; 0.64-1.59)	1.14 ± 0.48 (1.10 ; 0.71-1.54)	0.452[a]
CPB time	82.2 ± 23.7 (80.0; 63.3–94.0)	74.5 ± 18.5 (70.5; 60.0–84.0)	0.075[a]
ACC time (min)	49.1 ± 19.0 (45.5; 35.8– 60.2)	41.0 ± 12.9 (39.0; 30.0–50.2)	0.022[a]
Number of grafts	2.9 ± 0.9 (3;2–4)	2.7 ± 0.8 (3;2–3)	0.593[a]
Parameters of myocardial protection efficacy [7, 10]			
Postoperative MI	1 (1.8)	5 (10.0)	0.075[b]
Atrial fibrillation *de novo*	11 (20.4)	14 (28.0)	0.496[b]
Inotropic support	5 (9.3)	6 (12.0)	0.893[b]
cTnI > 8.5 μg/L/24 h	2 (3.7)	4 (8.0)	0.604[b]
Prolonged MV > 24 h	0 (0)	0 (0)	/
ICU-LOS (days)	2.8 ±1.8 (2.0; 2.0–3.0)	3.4 ± 4.3 (2.0; 2.0–3.0)	0.395[c]
Prolonged ICU-LOS > 3days	8 (14.8)	12 (24.0)	0.348[b]
Hospital (H) LOS (days)	7.7 ± 3.9 (7.0; 6.0–8.0)	8.0 ± 4.5 (7.0; 6.0–7.0)	0.811[c]
Prolonged H-LOS > 10 days	4 (7.4)	6 (12.0)	0.645[b]
30-day mortality	0 (0)	0 (0.0)	/
PMPE	20 (37.0)	28 (56.0)	0.053[b]

***Note*: Results are given as mean ± standard deviation (median; interquartile range) or number (percentage).**

LV – left ventricular; LVEF – LV ejection fraction (Simpson's method); CKD – chronic kidney diseases; GFR – glomerular filtration rate; Diseassed vessels – coronary stenosis > 70%; Euro score – European System for Cardiac Operative Risk Evaluation; MI – myocardial infarction; ICU – intensive care unit; LOS – length of stay; MV – mechanical ventilation;; PMPE – parameters of myocardial protection efficacy.

[a]*t* test; [b]χ^2 test; [c]Mann-Whitney U test.

surgery. Comparison between the groups in terms of PMPE showed a lower rate in the Custodiol® group than in the ST. Thomas M group (37.0% vs. 56.0%, respectively), which was very close to being statistically significant ($p = 0.053$) (Table 4).

Discussion

Myocardial injury is an inevitable consequence of cardiac surgery and cardioplegic arrest is the most preferable technique of intraoperative myocardial preservation. There have been numerous studies comparing the effectiveness of myocardial preservation between a wide variety of blood and crystalloid cardioplegia. However, their relative benefits are still a matter of an on-going debate [4, 8].

Our study compared two strategies of myocardial protection: one using Custodiol® and the other using modified St. Thomas cardioplegia. The difference between the studied groups according to the predictors of perioperative myocardial injury [6] and postoperative cTnI elevation [7] was not significant except in the case of aortic cross clamp (ACC) time and trimetizidine therapy. In particular, we observed a significantly longer ischemic period, with trends to significantly longer CBP time in the Custodiol® group and significantly higher perioperative use of trimetizidine a proven cardioprotective agent [9] in the St.Thomas group. Hypertrophied myocardium, which is much more difficult to protect, was considerably more common in the Custodiol® group, although without statistical significance. These could have an adverse effect on outcomes in the Custodiol® group.

Meta-analysis conducted by Edelman et al. [10] compared Custodiol® with other conventional crystalloid or blood cardioplegia, with regard to myocardial protection, summarizing fourteen comparative studies, and concluded that there was no difference in hospital mortality between Custodiol® and other conventional cardioplegia.

Our study showed no mortality, and the reason could be a low preoperative EuroScor II, and infrequent severe myocardial damage, according to relatively low cardiac enzymes levels. Spontaneous rhythm recovery after ACC is commonly used as an indicator of myocardial protection, ranging from 10% to 99% [11]. Meta-analysis by Edelman et al. [10] presented statistically significant higher rate of

ventricular fibrillation in Custodiol® groups (Custodiol® 20.1% vs. 9.7% in other groups). In our study spontaneous rhythm restoration rate was higher in the Custodiol® group, 31.5% vs. 20.0% in the St Thomas group, but without statistical significance ($p = 0.267$). No significant difference in the rates of postoperative MI, AF, low cardiac output with inotropic support between groups was found in our study, in agreement with the meta-analysis [10]. We observed five times more frequent MI in the Custodiol® group with a trend to statistical significance ($p = 0.075$). In the meta-analysis cross-clamp time was around 60 minutes in both groups and cardiac enzyme levels (CKMB and cTnI) did not differ between groups. The cross-clamp time in our study was under 50 minutes, with comparable enzyme levels between the groups.

There are only few studies in the literature that compared myocardial protection between Custodiol® and St. Thomas cardioplegia [12–16]. These studies discovered no significant difference between the two groups in terms of mortality, however, their findings on myocardial protection in terms of other specific indicators varied in a fashion which seems to suggest that benefits of Custodiol® cardioplegia becomes more pronounced with longer ACC. In the study by Arslan et al. [12], which involved shorter clamping time (less than 40 min), the only significant difference found was longer time to cardiac arrest in the Custodiol® group than in the St. Thomas group (63 vs. 54 seconds, respectively) that could be a disadvantage because it causes more ischemic-reperfusion damage [10, 12]. On the other hand, time to cardiac arrest in our study was about 60 seconds for both groups, whereas ACC time was about 40–50 min. Demmy et al. [13] demonstrated lower defibrillation rate after ACC (64% vs. 91%), but a significantly higher peak level of cTnI 6 hours after surgery in the Custodiol® group (no information about ACC time was given). Hamed et al. [14], in a study with ACC time mean value of about 60 min, showed that St. Thomas cardioplegia was comparable to that of Custodiol® and blood cardioplegia in pediatric cardiac surgery**.**

Careaga et al. [15] demonstrated improved myocardial protection in adult cardiac surgery with Custodiol® cardioplegia according to all considered indicators at myocardial ischemic time longer than 60 min. Lin et al. [16] demonstrated superiority of Custodiol® cardioplegia in complex pediatric cardiac surgery with ischaemic time over 150 min [16]. In our study cross-clamp time was below 50 min in both groups, although significantly longer in the Custodiol® group, and myocardial protection was comparable between the groups.

cTnI and CK-MB are routinely used to evaluate the degree of myocardial damage associated with cardiac surgery. cTnI has been shown to be the most sensitive biochemical marker of intraoperative myocardial injury and is therefore an valuable indicator of the quality of myocardial protection [7, 17]. It has shown that in uncomplicated cardiac surgery, there is an early increase of cTnI around 6 hours post surgery, followed by a rapid decrease, falling down to substantially lower concentrations at 24 hours. A later release of cTnI is more indicative of severe myocardial damage. The unfavorable outcome is indicated if cTnI peaked > 8.5 µg/L 24 hours post surgery [17–19]. Postoperative release of cardiac enzymes in our study over 48 hours, reached peaked values of around 5 µg/L at 6 hours post surgery in each group, indicating a lesser perioperative myocardial damage. This result is in accordance with previous studies [12–14]. PMPE variable revealed a higher rate of adverse outcomes of poor myocardial protection in the St.Thomas M group, very close to statistical significance ($p = 0.053$).

Conclusion

Custodiol® is safe and as effective as conventional cold crystalloid modified St.Thomas cardioplegia for myocardial protection in CABG surgery. The considerably less frequent MI, with a trend towards statistical significance, despite the significantly longer cross-clamp time in the Custodiol® group may suggest that its benefits even in operations with shorter ischemic time could be ascertained in a larger study.

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Possible risk factors for postoperative urinary tract infection following ureteroscopic lithotripsy

Mogući faktori rizika od nastanka postoperativne urinarne infekcije nakon ureteroskopske litotripsije

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Abstract

Background/Aim. Ureteroscopic lithotripsy is today a safe method of endoscopic destruction of stone in the kidney and ureter with a small number of complications of which the most common is postoperative urinary tract infection. Risk factors for the occurrence of urinary tract infection after the ureteroscopic destruction of stones in the ureter and kidney are not clearly defined in the previous studies. **Methods**. The study included 389 patients with ureteroscopic lithotripsy and possible risk factors were analyzed: age of the patients, sex, diabetes presence, degree of hydronephrosis, stone size, stone localization, wear of ureteral JJ stent and percutaneous nephrostomy catheter, type of surgical procedure and duration of the operation. The frequency of postoperative urinary tract infection was statistically analyzed in relation to the possible risk factors. **Results.** Ten percent of the patients had postoperative urinary tract infection. The higher incidence of postoperative urinary tract infection was found in patients with diabetes ($\chi^2 = 22.918$; $p < 0.001$), those who before surgery carried a ureteral JJ stent ($\chi^2 = 4.620$; $p = 0.040$) and percutaneous nephrostomy catheter ($\chi^2 = 8.240$; $p = 0.004$), who had a larger stone ($\chi^2 = -3.301$; $p = 0.001$), and whose surgery lasted longer ($t = 4.261$; $p < 0.001$). **Conclusion.** The frequency of postoperative urinary infection and risk factors for its emergence in our study are in line with the results of studies by other authors. Patients with diabetes, who preoperatively carried JJ stent or a percutaneous nephrostomy catheter, who had large stones and in which the operating time is longer have a greater risk of developing postoperative urinary tract infection. Accordingly, the importance of identifying these patients in the preparation for ureteroscopic lithotripsy contributes to the appropriate preoperative preparation and decreases the frequency of postoperative urinary tract infection to a minimum.

Key words:
ureteroscopy; lithotripsy; risk factors; infection postoperative complications.

Apstrakt

Uvod/Cilj. Ureteroskopska litotripsija je u današnje vreme sigurna metoda endoskopskog razbijanja kamena u bubregu i ureteru, sa malim brojem komplikacija, od kojih je najčešća postoperativna urinarna infekcija. Faktori rizika od pojave urinarne infekcije nakon ureteroskopskog razbijanja kamena u ureteru i bubregu nisu jasno definisani u dosadašnjim radovima. **Metode**. Istraživanjem je bilo obuhvaćeno 389 bolesnika kod kojih je urađena ureteroskopska litotripsija, a od mogućih faktora rizika analizirani su: starost bolesnika, pol, dijabetes, prisustvo i stepen hidronefroze, veličina kamena, lokalizacija kamena, nošenje ureteralne JJ sonde i perkutanog nefrostomskog katetera, vrsta operativne metode i dužina trajanja operacije. Statistički je bila analizirana učestalost postoperativne urinarne infekcije u odnosu na pomenute moguće faktore rizika. **Rezultati**. Postoperativnu urinarnu infekciju je imalo 10% bolesnika Veća učestalost postoperativne urinarne infekcije nađena je kod bolesnika koji su imali dijabetes ($\chi^2 = 22,918$; $p < 0,001$), koji su preoperativno nosili ureteralnu JJ sondu ($\chi^2 = 4,620$; $p = 0,040$) i perkutani nefrostomski kateter ($\chi^2 = 8,240$; $p = 0,004$), koji su imali veći kamen ($Z = -3,301$; $p = 0,001$) i kod kojih je operacija trajala duže ($t = 4,261$; $p < 0,001$). **Zaključak**. Učestalost postoperativne urinarne infekcije i faktori dijabetesa, koji preoperativno imaju postavljenu JJ sondu ili perkutani nefrostomski kateter, koji imaju veliki kamen i

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kod kojih je operativno vreme duže, imaju veći rizik od razvoja postoperativne urinarne infekcije. U skladu sa tim, značaj identifikacije ovih bolesnika u pripremi za ureteroskopsku litotripsiju doprinosi da se preduzme adekvatna preoperativna priprema i učestalost postoperativne urinarne infekcije smanji na najmanju meru.

Ključne reči:
ureteroskopija; litotripsija; faktori rizika; infekcija; postoperativne komplikacije.

Introduction

The endoscopic destruction of ureter and kidney stone or ureteroscopic lithotripsy (URSL) is a standard procedure in surgical treatment of urinary stones [1]. Previous studies have shown that ureteroscopic lithotripsy is a safe method with few complications and with a success rate of up to 85.6% [2]. The most common postoperative complications are infectious complications including transient febrile condition and urinary tract infection with a frequency of 1.7–18.8% [3–5]. Urosepsis is one of the most severe complications after ureteroscopic lithotripsy [6].

In previous studies that have been published, factors that are directly related and lead to infectious complications and sepsis are not clearly defined [7–10].

The importance of identifying possible risk factors for the occurrence of urinary tract infection after ureteroscopy because of ureter and kidney stones, either pneumatic or laser lithotripsy, would be crucial in order to take specific measures for the prevention of severe forms of postoperative urinary tract infection and sepsis [11, 12].

As individual risk factors for the development of urinary tract infections after ureteroscopic lithotripsy investigated so far were: age, sex, diabetes, bacteriuria and pyuria, acute pyelonephritis, the presence of percutaneous nephrostomy catheter and ureteral JJ stent, the presence of hydronephrosis, the use of antibiotic therapy before ureteroscopic lithotripsy, duration of surgery, the presence of kidney and heart diseases, the use of anticoagulant therapy, as well as the number, size and localization of the stone [13–15].

Methods

The study included 389 patients, 200 males and 189 females who underwent ureteroscopic lithotripsy with semi-rigid and/or flexible ureteroscope in a five-year period, from January 2010 to December 2014. The stone was fragmented using a laser with a power of 10 watts or pneumatic stone breaking device.

Preoperative clinical data included patient-related characteristics, stone characteristics, and type of procedures prior to surgery. Thus, preoperative clinical information regarding the patients included: age, sex, presence of diabetes, and degree of hydronephrosis.

Preoperative clinical data that were related to the stone were: stone size, stone localization, and presence of ureteral JJ stent and/or percutaneous nephrostomy catheter prior to surgery.

Surgery information included: the type of surgical procedure (pneumatic or laser lithotripsy with semi-rigid and/or flexible ureteroscope) and duration of the operation.

In all of the patients, the characteristics and degree of postoperative urinary tract infection were analyzed according to the modified Klavien-Dindo classification (MCCS) [16], and for the definition of urinary tract infection, we used provisions of the European Association of Urology (EAU) Section of Infection in Urology (ESIU) [17].

For the definition of sepsis, in addition to the criteria of ESIU, we used the provisions of the International Conference on the definition of sepsis and organ failure and guidelines for the use of innovative therapies in sepsis of American pediatricians and critical care organizations (Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis, The American College of Chest Physicians (ACCP)/Society of Critical Care Medicine (SCCM) Consensus Conference Committee, established in 1992 and supplemented 2001/2003 [18]. According to these criteria, sepsis is defined as the presence of the source of infection and Systemic Inflammatory Response Syndrome (SIRS). For SIRS, it is characteristic that two or more of the following criteria are present: body temperature > 38 ºC or < 36 ºC; heart rate > 90/min; respiration rate of 12/min or CO_2 partial pressure < 32 mmHg; leukocytosis: 12,000 or 4,000/mm^3. Severe sepsis is characterized by organ dysfunction and septic shock by acute circulatory collapse with persistent arterial hypotension [19].

All data in this study were processed in the SPSS 20.0 (IBM Corporation) software package. The selected level of significance or the probability of the first type error was 0.05. The examinees were classified into two groups. In the first group were patients who had ureteroscopic lithotripsy and did not have postoperative urinary tract infection. In the second group were patients with ureteroscopic lithotripsy who had postoperative urinary tract infection.

Results

Of the 389 patients who had ureteroscopic lithotripsy, 350 (90%) were without postoperative urinary tract infection and 39 (10%) were with postoperative urinary tract infection.

The average age of patients in the study was 55 (13–92) years. The average size of the stone was 13 (4–50) mm. In 94 (24.2%) patients lithotripsy was performed in the kidney, and in 295 (75.8%) in the ureter. According to kidney localisation, in the lower calyx lithotripsy was performed in 19 (4.9%) patients, in the middle calyx in 7 (1.8%), in the upper calyx in 2 (0.5%), and in the renal pelvis in 66 (17.0%) patients. According to ureter localization, lithotripsy in the lower ureter was performed in 68 (17.4%) patients, in the middle ureter in 94 (24.2%), and in the upper ureter in 133 (34.2%) patients. Laser lithotripsy was performed in 237 (60.9%) patients and lithotripsy with pneumatic probe in 152

Table 1

Incidence of infective complications and postoperative urinary infections and the treatment method

Complication	Patients n (%)	Treatment
Gradus I		
temporary febrile condition	34 (8.7)	Antipyretics
Gradus II		
SIRS	32 (8.3)	Antibiotic therapy
sepsis	3 (0.9)	Antibiotic therapy Parenteral infusion solution Inotropic drugs
Gradus III		
obstructive sepsis- pyelonephritis	2 (0.4)	Endoscopic intervention Placement of JJ stent or PNC
Gradus IVa		
severe sepsis	1 (0.2)	Intesive care unit management
Gradus IVb		
septic shock	1 (0.2)	Intesive care unit management

SIRS – Systemic Inflammatory Response Syndrome; PNC – percutaneous nephrostomy catheter.

(39.0%) ones. The laser was used for lithotripsy in the lower ureter in 15 (3.9%) patients, in the middle ureter in 44 (11.3%), in the upper ureter in 95 (24.4%), in the lower calyx in 19 (4.9%), in the middle calyx in 7 (1.8%), in the upper calyx in 1 (0.2%) and in the renal pelvis in 56 (14.4%) patients. Pneumatic probe was used in the lower ureter in 53 (13.6%) patients, in the middle ureter in 50 (12.9%), in the upper ureter in 38 (9.8%), in the upper calyx in 1 (0.2%) and in the renal pelvis in 10 (2.6%) patients. The average duration of the operation was 40 minutes. The shortest duration of the operation was 5 minutes, and the longest one 185 minutes. Semi-rigid ureteroscope was used in 357 (91.8%) patients, flexible in 28 (7.2%), and in 4 (1.0%) patients both types of ureteroscope were used. In all patients for the localization of stones in the ureter, only the semi-rigid instrument was used: in the lower ureter in 68 (17.4%), in the middle ureter in 94 (24.4%) and in the upper ureter in 133 (34.2%) patients. Semi-rigid instrument was used for lithotripsy in the renal pelvis in 61 (15.7%) patients and in the upper calyx in 1 (0.2%) patient. Flexible urethroscope was not used for lithotripsy in the ureter, but only in the kidney: in the lower calyx in 19 (4.9%) patients, in the middle calyx in 7 (1.8%), in the upper calyx in 1 (0.2%) and renal pelvis in 1 (0.2%) patient.

Infectious complications in our study were reported in 73 (18.7%) patients (Table 1). In 34 (8.7%) patients, a transient febrile state lasting up to 48 hours occured, and these patients, apart from the use of antipyretics, were not further treated. Postoperative urinary tract infection, according to the definitions of EAU/ESIU and Definitions for sepsis and organ failure and Guidelines for the use of innovative therapies in sepsis, the ACCP/SCCM from the International Sepsis Definitions Conference, had 39 (10%) patients [17–19]. In these patients, the treatment included the use of antipyretics, an additional antibiotic, infusion, inotropic and supportive therapy, and in 2 (0.4%) patients additional procedures were performed for placing an ureteral JJ stent and a percutaneous nephrostomy catheter.

A higher incidence of postoperative urinary tract infection was found in 32 (8.2%) patients who had diabetes ($\chi^2 = 22.918$; $p < 0.001$), in 48 (12.3%) patients who had an inserted ureteral JJ stent prior to surgery ($\chi^2 = 4.620$; $p = 0.040$) and in 52 (13.3%) patients who had a percutaneous nephrostomy catheter ($\chi^2 = 8.240$; $p = 0.004$) (Table 2). It was also reported in patients with larger stones ($Z = -3.301$; $p = 0.001$) and in patients in whom the operation lasted longer ($t = 4.261$; $p < 0.001$) (Table 3).

In our study no statistically significant difference was found between groups with and without postoperative urinary infection in relation to age, sex, hydronephrosis, the side where lithotripsy was performed, stone localization, type of ureteroscope, and type of stone fragmenting.

In all of the patients with postoperative urinary tract infections, an infective agent in the urinary culture was isolated. The most common bacterium was *Escherichia coli* (43.6%), followed by *Pseudomonas aeruginosa* (25.6%), *Klebsiella species* (12.8%), *Enterococcus faecalis* (5.1%), *Proteus mirabilis* (5.1 %), *Pseudomonas aeruginosa* + *Escherichia coli* (5.1%) and *Proteus mirabilis* + *Pseudomonas aeruginosa* + *Enterococcus faecalis* (2.7%).

Table 2

Diabetes, preoperatively inserted JJ stent and percutaneous nephrostomy catheter (PNC) as possible risk factors for predicting postoperative urinary tract infection following ureteroscopic lithotripsy

Parameter	Total n (%)	Postoperative urinary tract infection, n (%)		*p*-value
		No	Yes	
Number of patients	389 (100)	350 (90.0)	39 (10.0)	
Diabetes	32 (8.2)	21 (65.6)	11 (34.4)	< 0.001
Preoperatively inserted JJ stent	48 (12.3)	39 (81.2)	9 (18.8)	0.040
PNC	52 (13.3)	41 (78.8)	11 (21.2)	0.004

Table 3
Stone size and operative time duration as possible risk factors for predicting postoperative urinary tract infection following ureteroscopic lithotripsy

Postoperative urinary tract infection	Number of patients	Stone size* (mm) mean (range)	Operative time duration (min) mean (range)
No	350	12.7 (4–50)	42.6 (5–185)
Yes	39	16.1 (5–35) †	60.0 (20–130)†
Total	389	13.2 (4–50)	44.4 (5–185)

***Stone size was calculated as the sum of the diameter of each stone in case of multiple stones.**
† $p < 0.001$.

Discussion

This study examined some of the possible risk factors for the occurrence of postoperative urinary tract infection in patients following ureteroscopic lithotripsy.

Although ureteroscopic lithotripsy is a safe procedure today, the risks of occurring postoperative infectious complications are not negligible [14] and are not clearly defined in the existing literature [1].

Diabetes mellitus is associated with older age and other chronic diseases. Daels et al. [20] analyzed the data from the Clinical Research Office of the Endourological Society (CROES) database of a multicentre study of Endourological Society, which included 114 hospitals from 32 countries and 11,885 patients with ureteroscopic lithotripsy, and concluded that the risk of complications was greater in elderly patients suffering from associated diseases. It was found that there was a significant risk of complications in patients suffering from cardiovascular disease, diabetes, obesity and patients using anticoagulant therapy. In many studies, diabetes was analyzed as a risk factor for the occurrence of infectious complications following ureteroscopic lithotripsy. Uchida et al. [10] analyzed diabetes and frequency of SIRS following ureteroscopic laser lithotripsy but did not find statistically significant difference between groups without and with postoperative signs of SIRS ($p = 0.71$) [10]. Similar results were published by Berardinelli et al. [16], Moses et al. [7], and Sohn et al. [14] in their papers. However, in our study of 32 patients with diabetes, who had ureteroscopic lithotripsy, 11 (34.4%) patients had a postoperative urinary tract infection. By univariate analysis, it was concluded that there was a statistically significant difference between groups without and with postoperative urinary tract infection ($p < 0.001$). Diabetes as a risk factor for postoperative urinary tract infection was examined by Martov et al. [21] in 2015. They analyzed data from the CROES database and concluded that in patients with diabetes the incidence of postoperative infections was higher ($p < 0.05$).

By multivariate analysis in our study, preoperatively placed ureteral JJ stent represented a significant risk factor for the occurrence of postoperative urinary tract infection ($p = 0.024$). A JJ stent was preoperatively placed due to verified obstructive pyelonephritis in 48 (12.3%) patients. It was left in place during an average 4 weeks, and in the group with postoperative urinary infection for 8 weeks. Also, by univariate analysis, statistically significant difference existed between groups without and with postoperative urinary infection in relation to preoperatively placed JJ stent ($p = 0.040$). In a study by Japanese authors, Mitsuzuka et al. [22], preoperatively placed JJ stent was associated with a higher incidence of postoperative febrile urinary tract infection in the univariant ($p = 0.013$), but not in multivariate analysis ($p = 0.529$). In this study, the univariate ($p < 0.001$) and multivariate ($p = 0.044$) analyses showed that acute pyelonephritis was a significant risk factor for the occurrence of postoperative urinary infection. Half of the patients with JJ stent had preoperatively acute pyelonephritis, so it was not entirely clear in what degree the preoperatively placed JJ stent independently influenced the occurrence of postoperative urinary infection. The presence of preoperative JJ stent was associated with the appearance of SIRS in a study by Uchida et al. [10], which was proven by the univariate analysis ($p < 0.001$), but not by multivariate analysis. There is possible bacterial colonization on the surface of the stent (bacterial "biofilm"), and due to the reflux of urine from the urinary bladder, the risk of pyelonephritis and sepsis increases [11]. In a study published by Moses et al. [7] in 2016, 550 patients underwent ureteroscopic laser lithotripsy and in 327 (60%) patients, a JJ probe was placed for passive dilatation of the ureter. Postoperative urinary tract infection was more frequent in the group with JJ stent ($p = 0.025$). However, these results are in contrast to those from a study published by Blackmur et al. [12] and show that preoperatively placed JJ stent reduces the risk of postoperative SIRS in patients with preoperative positive urine culture.

Percutaneous nephrostomy catheter, as well as ureteral JJ stent, is most often preoperatively placed in patients with obstructive pyelonephritis. In our study, 52 (13.4%) patients had percutaneous nephrostomy (PNS) catheter prior to ureteroscopic lithotripsy. PNS catheter was necessarily placed in a patient who had a preoperatively verified hydronephrosis of 3rd or 4th grade according to the 2007 classification by Onen [23]. In case of suspicion of obstructive pyelonephritis, renal failure, and renal impairment, PNS catheter is installed regardless of the degree of hydronephrosis. The univariate analysis showed that patients with preoperatively established PNS catheter recorded a higher incidence of postoperative urinary tract infection ($p = 0.004$), which is in line with a study published by Sohn et al. [14], in which patients who had preoperatively PNS catheter had a greater incidence of infectious complications. However, in a study by Japanese authors, Uchida et al. [10], in

patients with preoperatively introduced PNS catheter, no higher incidence of postoperative infectious complications was found ($p = 0.42$). These authors state that PNS catheter plays an important role during ureteroscopic lithotripsy improving intraoperative irrigation and reducing intrarenal pressure. This discrepancy between studies, and higher incidence of postoperative urinary tract infection in our study and in that by Sohn et al. [14], can also be explained by the fact that patients who had PNS catheter prior to surgery were mainly patients with multiple stones, big stone and preoperative bacteriuria (due to colonization of PNS catheter surface with bacterial biofilm), which also had an effect on the occurrence of postoperative urinary tract infection.

The size of the stone in our study was from 4 to 50 millimeters. In patients who had multiple stones, but at one localization, the total size of the stone was taken as the sum of diameters of all the stones. Uchida et al. [10] analyzed the cumulative volume of the stone as the volume of the each stone [cumulative diameter of the stone in their study amounted to 10 millimeters (3–47 millimeters)] and did not find that the volume and size of the stone were significant factors for the occurrence of postoperative urinary tract infection. In our study, in the group of patients with postoperative urinary tract infection, the mean stone size was 16.1 millimeters, and in the group of patients who did not have a postoperative urinary infection, the mean stone size was 12.7 millimeters. These results might be an explanation for the statistical significance of the occurrence of postoperative urinary tract infections after ureteroscopic lithotripsy ($p = 0.001$) in our study in relation to results published by Uchida et al. [10] since the cumulative stone diameter in our study was higher. In a study from 2015, Mitsuzuka et al. [22] analyzed the stone size as a risk factor for the occurrence of postoperative urinary tract infection, and no statistical significance of this parameter was found ($p = 0.139$). In their study, the stones were divided by sizes into stones smaller than 20 millimeters and those of 20 or more millimeters. In a group of patients with a stone size below 20 millimeters, 15.2% of patients had postoperative urinary infection, and 25% of patients had postoperative urinary infection when the stone sizes were 20 or more than 20 millimeters.

The average length of surgery in our study was 44.4 (5/185) minutes, and in the group of patients with postoperative urinary infection 60 (20/130) minutes. Multivariate analysis showed that there was a statistically significant difference between groups without and with postoperative urinary tract infection in relation to the length of the operation ($p < 0.001$). It is understandable that the length of ureteroscopic lithotripsy may also depend on the severity of the case, the technical deficiencies of the existing equipment, the stone size, as well as the stone type and location. The length of the operation increases the intraoperative exposure to the bacteria that are located on the surface of the stone or are released from the stone breaking. Knipper et al. [24] found in their study that longer operating time was associated with complications. Moses et al. [7] state that if the operative time lasts over 120 minutes, it is associated with postoperative urinary tract infection ($p < 0.001$). The identical results are found by Fan et al. [25] ($p = 0.026$) and Martov et al. [21] ($p < 0.001$), who in their studies suggest that the length of the operation has an effect on the higher incidence of postoperative urinary tract infection. However, Berardinelli et al. [16], Mitsuzuka et al. [22], and Sohn et al. [14] state that the length of the operation did not cause the higher incidence of postoperative urinary infection. The explanations for the difference of our results with the results of Berardinelli et al. [16], Mitsuzuka et al. [22], and Sohn et al. [14] studies are that in those studies the total diameter of the stone was smaller. Also, there was a higher number of patients in our study who had a preoperatively and postoperatively placed ureteral JJ stent, which prolonged the duration of the operation and exposure to bacteria.

Infectious complications in our study were reported in 73 (18.7%) patients. Postoperative urinary infection, as defined by the EAU/ESIU and Definitions for sepsis and organ failure and Guidelines for the use of innovative therapies in sepsis, the ACCP/SCCM, from the International Sepsis Definitions Conference, had 39 (10%) patients. According to these criteria, all 39 patients had complicated postoperative urinary tract infection [all had an elevated body temperature ($\geq$ 38 ºC) and leukocytosis (12,000/mm^3] and their treatment required the use of antipyretics, an additional antibiotic therapy, infusion, inotropic and supportive therapy. In 2 (0.5%) patients, additional procedures for placing JJ stent and percutaneous nephrostomy catheter were made.

In our study, 34 (8.7%) patients had a transient febrile state lasting up to 48 hours and defined according to the MCCS as grade I complications. Infectious of grade I complications in our study were not classified as postoperative urinary tract infection because patients did not require additional pharmacological treatment or the use of antibiotic therapy, except for the use of antipyretics. Mitsuzuka et al. [22] stated that febrile status following ureteroscopic lithotripsy occurred in 15% of patients, and the total number of infectious complications after ureteroscopic lithotripsy in their study was 18.3%. As in our study, Mitsuzuka et al. [22] classified febrile condition with a body temperature up to 38 ºC, without the need for additional treatment or administration of antibiotics as an infectious complication of grade I.

Out of 39 patients with postoperative urinary tract infections in our study, 35 (8.9%) patients had grade II infectious complications, of which 32 (8.3%) had characteristic signs for SIRS (with a measured body temperature of 38ºC and leukocytosis over 12,000). Uchida et al. [10] reported 5.7% of patients with SIRS following ureteroscopic laser lithotripsy. Out of 27 patients who had SIRS in our study, the condition of one patient required admission to the intensive care unit, but no fatal outcome was registered. This indicates that SIRS does not necessarily lead to a fatal outcome, but requires a long treatment that has an impact on the physical and economic status of a patient. Of 35 patients in our study with grade II infectious complications, 3 (0.9%) patients developed sepsis, which

was confirmed by a positive, hemoculture. These 3 patients, besides signs for SIRS had hypotension and cardiovascular collapse and required treatment with an additional antibiotic therapy and use of infusion solutions and inotropic drugs, but their condition did not require staying in the intensive care unit, although the finding of hemoculture was positive. In all 3 patients, *Staphylococcus coagulase* (-) was isolated. In 2 (0.5%) patients with grade III complications, obstructive pyelonephritis and sepsis were postoperatively verified, and apart from the application of antibiotic therapy, it was necessary to place JJ stent in one patient, and percutaneous nephrostomy catheter in the second one. In one patient, *Escherichia coli* was isolated from the hemoculture, and in the second one *Staphylococcus coagulase* (-). Two patients in our study had grade IV complications and were treated in the intensive care unit due to circulatory collapse and cardiorespiratory dysfunction, under the diagnosis of severe sepsis and septic shock. They were intubated and ventilated, with simultaneous administration of several antibiotics, inotropic drugs, and colloidal and nutritional solution infusions. *Escherichia coli* was isolated from hemoculture in both patients.

Sepsis after ureteroscopic lithotripsy is one of the most severe complications. In our study, 7 (1.8%) patients developed clinical signs of the sepsis. Out of this number, 5 (1.4%) patients were treated in the department, and 2 (0.4%) required monitoring and treatment in the intensive care unit. In other studies that dealt with risk factors for the occurrence of postoperative urinary tract infection, the frequency of sepsis was between 1–3%. Mitsuzuka et al. [22] reported 1.3% of patients with sepsis following ureteroscopic lithotripsy. In the existing literature, only a few studies analyzed the frequency of sepsis following ureteroscopic lithotripsy. Geavlete et al. [26] reported that 1.13% of 2,735 patients had sepsis after ureteroscopic lithotripsy with a semi-rigid ureteroscope. Eswara et al. [15] analyzed 328 patients who had endourological procedures, of which 11 (3%) had sepsis. However, Blackmur et al. [12], in their analysis of the risk factors for the occurrence of sepsis after ureteroscopic lithotripsy in 462 patients, reported that 34 (7.4%) patients had sepsis. This somewhat larger number of patients who had sepsis after ureteroscopic lithotripsy can be explained by the fact the study included both patients with bilateral ureteroscopic lithotripsy and a large number of patients who had associated cardiovascular disease and diabetes, a high American Society of Anestesiology (ASA) score and a larger volume of stones.

Our study had several shortcomings and limitations. All of 389 patients were referred for treatment from smaller hospitals and they already had a complicated state with large or infected stone since our institution is a tertiary reference center for the treatment of urolithiasis. Most of these patients had associated comorbidities, and previously failed procedures in other hospitals. These were the possible reasons for a greater incidence of postoperative urinary tract infections following ureteroscopic lithotripsy in our study than in reference ones. Additional intraoperative urinalysis for bacterial examination and bacteriological examination of stones and fragments obtained during the procedure, that could provide additional information in the selection of antibiotics for the prevention of severe infectious complications, were not done in this study.

However, despite some shortcomings, the benefits of the study are: the broad age group of patients who were analyzed, the evaluation of a large number of variables and the use of standardized criteria in the identification of risk factors for the emergence of postoperative urinary tract infection after ureteroscopic lithotripsy. Also, the use of a standardized classification system for infectious complications (MCCS) made it easier and more accurate for comparison with reference studies.

Conclusion

Patients with diabetes, preoperatively placed JJ stent or percutaneous nephrostomy catheter, large stones and with prolonged operating time, have a higher risk of developing postoperative urinary infection. Accordingly, adequate preoperative preparation and antibiotic prophylaxis can contribute to preventing infectious complications and postoperative urinary tract infection in these patients.

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The influence of temporary occlusion of parent vessel on outcome of surgical treatment of ruptured cerebral aneurysms

Uticaj privremene okluzije nosećeg krvnog suda na rezultat hirurškog lečenja rupturiranih cerebralnih aneurizmi

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Abstract

Background/Aim. Aneurysm rupture followed by subarachnoid or intracerebral haemorrhage is always current topic and poses a great challenge to neurosurgeons. The aim of the study was to establish whether applying temporary occlusion before placing a final clip was justified. **Methods.** A prospective study was conducted on patients with aneurysm rupture, treated at Neurosurgical Clinic in Niš from January 2012 to December 2016. Patients belonging to I and II and 1, 2 and 3 grades according to the Hunt-Hess grading system and Fisher scale, respectively, were monitored. **Results**. In 85, out of total 182 bleeding aneurysms that were treated, a neurosurgeon decided to apply temporary clipping before placing the final clip. Temporary occlusion significantly influenced the presence of resulting neurological deficit. **Conclusion.** The application of temporary occlusion facilitates placing the final clip but also affects the occurrence of neurological deficits. It is assumed that this is a consequence of caused vasospasm, considering that these are bleeding aneurysms.

Key words:
intracranial aneurysm; aneurysm ruptured; therapeutic occlusion; neurosurgical procedures; vascular surgical procedures; treatment outcome.

Apstrakt

Uvod/Cilj. Ruptura aneurizme praćena subarahnoidalnom ili intecerebralnom hemoragijom je uvek aktuelna i predstavlja veliki izazov za neurohirurge. Cilj rada je bio da se ustanovi da li je opravdana primena privremene okluzije pre plasiranja definitivnog klipa. **Metode.** Sprovedena je je prospektivna studija u koju su bili uključeni bolesnici kod kojih je došlo do rupture aneurizme, lečeni na Neurohirurškoj klinici u Nišu, u periodu od januara 2012. do decembra 2016. godine. Bolesnici su po Hunt –Hess-ovoj gradaciji pripadali I i II, a po Fisher-ovoj skali 1, 2 i 3 stepenu gradacije. **Rezultati.** Od ukupno rešavane 182 krvareće aneurizme, kod njih 85 hirurg se odlučio da primeni privremeno klipovanje pre definitivnog plasiranja klipa. Privremena okluzija imala je značajan uticaj na prisustvo rezultirajućeg neurološkog deficita. Primena privremene okluzije značajno je uticala na prisustvo nastalog neurološkog deficita. **Zaključak.** Primena privremene okluzije olakšava plasiranje definitovnog klipa, ali utiče i na pojavu neurološkog deficita. Pretpostavlja se da je to posledica izazivanog vazospazma, uzimajući u obzir da se radi o krvarećim aneurizmama.

Ključne reči:
aneurizma, intrakranijalna; aneurizma, ruptura; okluzija, terapijska; neurohirurške procedure; hirurgija, vaskularna, procedure; lečenje, ishod.

Introduction

Aneurysms are a distention to the brain blood vessels caused by the weakness of the blood vessel wall. The spots where arteries branch off are the most common places in which aneurysms can be found. Over time, under the influence of blood wave in the blood vessel itself, the wall is getting increasingly thinner and the distention becomes sac-shaped (saccular distention) or spindle-shaped (fusiform distention). Such an aneurysm does not give any symptoms. It is usually detected as a sporadic finding during the examination. A serious manifestation of an aneurysm is its rupture. The annual incidence of bleeding is 10–14/100.000; 15–20% aneurysms bleed in the course of life, most often between the age of 40 and 60 [1].

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According to most literature data, total aneurysm-related mortality due to subarachnoid bleeding is 32–67%, decreasing with the advancement of therapy in the last three decades by about 0.5% per year [2–4]. Studies also say that gender does not affect the patient's outcome after aneurysm rupture [4, 5–7].

The symptoms of bleeding aneurysm are a severe headache accompanied by nausea and vomiting, as well as impaired consciousness. The depth of consciousness impairment depends on the magnitude of aneurysm bleeding. Usually, soon after rupture, it is temporarily spontaneously closed by a coagulum. The patient's condition stations at a certain level and then diagnostics can be approached. The method of choice today is brain multislice computed tomography (MSCT) with angio scan revealing both the existence of subarachnoid hemorrhage or intracerebral hematoma and the site of aneurysmal enlargement on the blood vessel. A surgeon decides whether aneurysm diagnosis using MSCT angio scan is sufficient or it is necessary to approach digital subtraction angiography (DSA). This diagnostic method enables a better display of localization, size and especially the position of the aneurysm neck in relation to the blood vessel. Diagnosis is followed by aneurysm treatment with two methods used in practice, surgical treatment by aneurysm clipping, or endovascular closing of enlargement using spirals. In this paper, we focused on the surgical way of treating a bleeding aneurysm and on the frequent surgeon's dilemma whether to apply a temporary occlusion before the final clipping of an aneurysm or not. Elective temporary occlusion in the treatment of intracranial aneurysms was first performed by Jefferson in 1928. Temporary clamping and moderate hypothermia in the treatment of aneurysms were reported by Suzuki et al. [8] in 1969. The authors pointed that intermittent reperfusion allowed prolongation of the total time of temporary occlusion. The technique of safe clipping as generally used depends on the temporary occlusion of the cerebral vasculature during surgery. It may lessen the risk of intraoperative aneurysm rupture and also allows evacuation of intramural calcification and thrombosis before definitive clipping in large aneurysms [9].

Methods

A prospective study was conducted on patients with aneurysm rupture, treated at the Neurosurgical Clinic in Niš, in the period from January 2012 to December 2016. According to initial clinical status, we could grade patients using the Hunt-Hess scale, and, according to their brain CT scan findings they were graded by using the Fisher scale. Patients who belonged to I and II and 1, 2 and 3 grades by the Hunt-Hess and Fisher scales, respectively, were monitored. The study covered a total of 182 patients who were surgically treated for aneurysmal change in brain blood vessels. Patients were all of the cases initially postoperatively treated in the Intensive Care Unit. All of the patients received anti-edematous therapy, analgesics before and after the operation.

Results

Out of total 182 bleeding aneurysms treated, in 85 a surgeon decided to apply temporary clipping before placing the final clip (Figure 1).

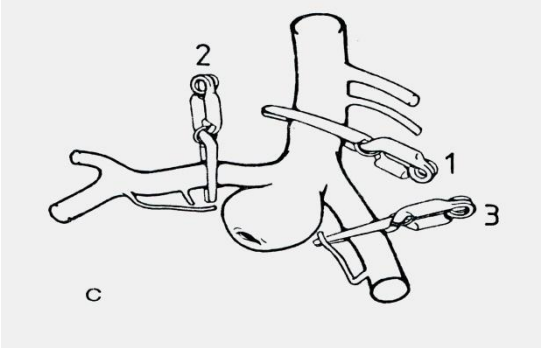

Fig. 1 – The application of a temporary occlusion.

As far as the localization of treated aneurysms was concerned, no statistical significant difference was found in relation to the application of temporary clipping before the final clip placement ($\chi^2 = 0.632$; $p = 0.959$), (Table 1).

Ischemic changes diagnosed using the brain MSCT with associated neurological deficit were recorded in 12 (12/85; 14.1%) of the patients treated with temporary occlusion while the same was present in 8 (8/97; 8.2%) of the patients with no temporary occlusion.

The application of temporary occlusion caused significantly higher incidence of neurological deficit in comparison with the treatment with no use of temporary clipping ($\chi^2 = 8.801$; $p = 0.003$), (Table 2).

The lethal outcome occurred in 7 (7/85, 8.2%) of the

Table 1

Localization of surgically treated aneurysms

Aneurysm localisation	With applied temporary occlusion	Without applied occlusion	χ^2	p
Arteria carotis	10	12		
Arteria cerebri posterior	8	12		
Arteria cerebri anterior	5	5	0.632	0.959
Arteria cerebri media	38	44		
Arteria communicans antrior	24	24		
Total	85	97		

Table 2

Neurological deficit

Ischemic changes	With applied temporary occlusion, n (%)	Without applied occlusion, n (%)	χ^2	*p*
No	73 (85.9)	89 (91.8)	8.801	0.003
Yes	12 (14.1)	8 (8.2)		
Total	85 (100)	97 (100)		

Table 3

Lethal outcome

Lethal outcome	With applied temporary occlusion, n (%)	Without applied occlusion, n (%)	χ^2	*p*
No	78 (91.8)	71 (73.2)	10.522	0.001
Yes	7 (8.2)	26 (26.8)		
Total	85	97		

Table 4

Duration of temporary occlusion

Duration (min)	Resulting ischemia, n (%)	Lethal outcome, n (%)	χ^2	*p*
Up to 3	0 (0)	0 (0)		
4–10	4 (33.3)	2 (28.6)	0.046	0.829
Over 10	8 (66.7)	5 (71.4)		
Total	12 (100)	7 (100)		

patients with applied temporary occlusion while in the group without temporary occlusion, 26 (26/97, 26.8%) of the patients succumbed to the effects of the intervention.

The lethal outcome was significantly more frequent among patients without applied temporary occlusion (χ^2 = 10.522; p = 0.001), (Table 3).

The duration of temporary occlusion did not significantly affect the final outcome of the patients treated (χ^2 = 0.046; p = 0.829) (Table 4).

Discussion

An early surgical procedure involving aneurysmal change clipping and basal cisterns blood clearing is a significant therapeutic procedure in preventing vasospasm occurrence, as shown by other authors [10, 11].

Surgical treatment of brain blood vessel aneurysms requires a rich experience, precision and good manual capability of an operator. To well observe the aneurysmal change, preserve all the surrounding branches, do good neck preparation and prepare it for putting the final clip require serious and painstaking work. Bleeding aneurysms are especially difficult to manage due to the condition of the very brain mass that is swollen, tense, prone to frequent bleeding, preventing the preparation of supply vessels and access to the very aneurysmal change.

Roganović et al. [12] described the main artery occlusion in 4 patients and in 3 more patients the posterior communicating artery was asymptomatically occluded along with the aneurysm. *Arteria carotis interna* (ACI) was occluded in two patients while distal *arteria cerebri anterior* (ACA) segment (parasagittal frontal and frontobasal infarction) and the final branch of *arteria cerebri media* (ACM) (temporoparietal infarction) were occluded in one patient, each. The risk of re-rupture in the course of the work is also high. Postoperative complications in the form of incomplete closure of the aneurysm, perforator occlusion, occlusion (subocclusion) of the main artery stem are also present. It is suggested that another clip should be previously placed distally in relation to the aneurysm in order to prevent filling of the fundus [13, 14].

During our research, out of the total 182 bleeding aneurysms, in 85 of them the operator decided to apply temporary clipping before placing the final clip. As far as the localization of treated aneurysms was is concerned, no statistical significance was found in relation to the application of temporary clipping before the final clip placement (χ^2 = 0.632; p = 0.959).

Placement of a temporary clip on the supply blood vessel with an aneurysm enables reduced blood flow through the blood vessel and therefore through the aneurysm. Aneurismal change volume reduces making the aneurysm easier for manipulation and enabling better preparation and separation of the surrounding branches. In the case of aneurysm rupture, the blood quantity is lower and under lower pressure, therefore clip placement is facilitated. Our study showed that, compared to the surgical treatment of ruptured cerebral aneurysms without previous temporary clipping, the application of temporary occlusion significantly reduced the presence of the resulting neurological deficit (χ^2 = 8.801; p = 0.003). Similarly, the lethal outcome was significantly more frequent among patients who were not treated with temporary occlusion (χ^2 = 10.522; p = 0.001). Some studies have shown that postoperative complications in terms of ischemic lesions and neurologic defects are associated with the duration of temporary occlusion [15, 16]. However, studies of other authors have not shown that there is a connection between the duration of occlusion and the occurrence of ischemia [17, 18]. During our study, we also did not record the effect of temporary occlusion duration on the

outcome of treated patients (χ^2 = 0.046; p = 0.829). Also, independently of placing temporary occlusion, vasospasm can occur as a postoperative complication leading to a neurological deficit and lethal outcome. Development of ischemia depends on following factors: reduction of blood vessel lumen by at least 50%, blood pressure values, intracranial pressure and blood viscosity. Also, atherosclerotic changes and levels of oxygen, carbon dioxide and haemoglobin in blood significantly influence the occurrence of vasospasm. The length of the stenosis caused by vasospasm and the quality of anastomoses on the base and in the Circle of Willis are also significant [19]. Contrary to this, the research of Malinova et al. [20] has shown that the use of temporary occlusion does not lead to provocation of vasospasm and these authors believe that one should not hesitate in using elective temporary clipping if it is considered appropriate.

In our opinion, surgical treatment of ruptured cerebral aneurysms requires extensive experience, knowledge, precision, and despite the effort made, the prognosis of healing is uncertain. There still remains a dilemma on whether it is desirable to place a temporary clip.

Conclusion

The application of temporary occlusion in the treatment of ruptured cerebral aneurysms enables easier placement of the final clip. The application of temporary occlusion influence the presence of the resulting neurological deficit. There still remains a dilemma: to apply temporary occlusion or not? Our research has shown that it is necessary to avoid the use of elective temporary occlusion, but if the situation requires, it is not disputed to apply it. It is assumed that it may be one of the causes of vasospasm provocation, considering that these are bleeding aneurysms.

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Association between ultrasonographically measured visceral fat tissue thickness and proinflammatory adipokines in obesity

Odnos ultrazvučne debljine visceralnog masnog tkiva i proinflamatornih adipokina u gojaznosti

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Abstract

Background/Aim. Obesity status can be assessed with numerous anthropometric, morphological and functional indices and this study was designed to assess relationship among them. The aim of this study was to investigate associations between anthropometric indices, ultrasonography measurement of visceral and subcutaneous fat tissue thickness and certain proinflammatory adipokines level. **Methods.** This cross-sectional study comprised a consecutive sample of 60 obese respondents without obesity-related comorbidities, and 20 age-matched healthy normal-weight controls. Anthropometric [body mass index (BMI), waist circumference (WC), neck circumference (NC), body fat, a body shape index (ABSI)], and ultrasonographic indices [thickness of intraabdominal fatt tissue (IAFT), visceral fat (VF), maximum subcutaneous fat (Max SFT), minimal subcutaneous fat (Min SFT)], and serum levels of chemerin and resistin were assessed in all subjects. **Results.** All anthropometric indices showed statistically significant differences between study groups. The mean IAFT, Max SFT, Min SFT and VF were significantly higher in the obese group compared to controls ($p < 0.01$, for all). Serum levels of chemerin and resistin correlated positively with BMI, percentage of fat adipose tissue (FAT, %), total FAT (kg), and VF ($p < 0.05$, for all). Also, we observed significant correlation between resistin and NC ($r = 0.23$, $p = 0.03$) and ABSI ($r = 0.22$, $p = 0.04$). In multivariable linear regression analysis, chemerin ($\beta = 0.23$; $p = 0.008$) and resistin ($\beta = 0.43$; $p = 0.002$) were independently and significantly associated only with VF. **Conclusion.** Obesity indices, both classical and newer ones, are in positive, statistically significant correlation with the level of proinflammatory cytokines. Ultrasonographically measured VF thickness, independently associated with adipokine levels, may improve assessment of proinflammatory fat tissue characteristics. Further studies are needed to precisely define the use of ultrasonographic fat tissue measurements into clinical practice.

Key words:
adipokines; adipose tissue; anthropometry; metabolic diseases; obesity; resistin; severity of illness index; ultrasonography.

Apstrakt

Uvod/Cilj. Stepen gojaznosti može se proceniti brojnim antropometrijskim, morfološkim i funkcionalnim indeksima, te je ova studija dizajnirana da ispita međusobni odnos ovih parametara. Cilj studije bio je da ispita povezanost antropometrijskih indeksa, ultrasonografskih merenja debljine potkožnog i visceralnog masnog tkiva i nivoa pojedinih proinflamatornih adipokina. **Metode.** Ispitivanjem je obuhvaćeno 60 gojaznih osoba, bez prisustva komorbiditeta vezanih za gojaznost i 20 normalno uhranjenih ispitanika usklađenih po polu i godinama. Antropometrijski pokazatelji [indeks telesne mase (BMI), obim struka (WC), obim vrata (NC), masa i procenat masnog tkiva [FAT (kg, %)], indeks oblika tela (ABSI)], ultrazvučna merenja [intra-abdominalno masno tkivo (IAFT), visceralno masno tkivo (VF), maksimalno potkožno masno tkivo (Max SFT), minimalno potkožno masno tkivo (Min SFT)], kao i serumski nivoi hemerina i rezistina određivani su kod svih ispitanika. **Rezultati.** Kod svih antropometrijskih pokazatelja uočena je statistički značajna razlika među ispitivanim grupama. Ultrazvučni pokazatelji masnog tkiva (IAFT, VF, Max SFT, Min SFT) bili su statistički značajno viši u grupi gojaznih osoba u poređenju

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sa kontrolnom grupom ($p < 0,01$ za sve). Serumski nivoi hemerina i rezistina pozitivno su korelisali sa BMI, FAT(%), FAT (kg) i VF ($p < 0,05$ za sve). Takođe, uočena je značajna korelacija između rezistina, sa jedne i NC sa druge strane ($r = 0,23$; $p = 0,03$), kao i ABSI ($r = 0,22$; $p = 0,04$). Multivarijabilna regresiona analiza pokazala je nezavisnu i značajnu povezanost hemerina ($\beta = 0.23$; $p = 0.008$) i rezistina ($\beta = 0.43$; $p = 0.002$) samo sa VF. **Zaključak.** Pokazatelji gojaznosti, kako klasični, tako i noviji, pozitivno i statistički značajno korelišu sa nivoom proinflamatornih adipokina. Debljina visceralnog masnog tkiva, merena ultrazvukom, nezavisno povezana sa nivoima adipokina, može poboljšati procenu karakteristika proinflamatornog masnog tkiva. Dalje studije su potrebne kako bi se precizno definisala upotreba ultrazvučnih mera masnog tkiva u kliničkoj praksi.

Ključne reči:
adipokini; masno tkivo; antropometrija; metaboličke bolesti; gojaznost; rezistin; bolest, indeks težine; ultrasonografija.

Introduction

Obesity is a disease characterized by increased fatty body mass in a degree that leads to health deterioration and development of numerous complications [1]. Obesity is very complex, with characteristics of global epidemic and if this trend continues, by 2030 almost about 85% of adult population will be obese and preobese [2]. Heterogeneous predisposing factors have significant impact on obesity occurrence, comprising genetic factors, childhood obesity – "fat cell theory", as well as extrinsic factors including sedentary lifestyle, unhealthy diet and low physical activity [3].

Numerous anthropometric measurements [body mass index (BMI), waist circumference (WC), hip circumference (HC), and their ratio – waist/hip ratio (WHR)] are being used for both obesity assessment and monitoring the effects of obesity therapy [4]. Emerging anthropometric parameter is a body shape index (ABSI), which proved to be superior to BMI in prediction of cardiometabolic diseases [5, 6].

Aside from anthropometric parameters, imaging techniques have a significant role in visualization and measurement of fat tissue compartments. Computed tomography (CT) and magnetic resonance imaging (MRI) are golden standard in fat tissue evaluation, however they present numerous limitations (machines, radiation exposure, length of examination, price). Ultrasonographic measurement of visceral and subcutaneous fat tissue compartments, as well as hepatic fat content, proved to be simple and reliable method in cardiometabolic risk evaluation, more precise and reproducible compared to WC, HC and WHR [7–9].

Increased secretion of proinflammatory adipokines is one of the most important characteristics of dysfunctional fat tissue. They are related to numerous pathophysiological processes which are baseline of some chronic disorders (low degree chronic inflammation, insulin resistance, arterial hypertension, dyslipidemia) as well as cardiometabolic, malignant and other diseases. It is known that resistin originates from adipocytes, immune cells and endothelial cells, and has significant role in processes of promotion of proinflammatory adhesive molecules and intracellular adhesive molecules expression and decreasing antiinflammatory effects of adiponectin on endothelial vascular cell [10]. The role of chemerin is also significant, although not sufficiently researched up to now. Chemerin is a growth factor, chemokine and adipokine, and is predominantly secreted in fatty tissue, and its expression is present in other tissues, such as liver, placenta, thrombocytes and kidneys [11, 12]. Contemporary researches emphasize the role of chemerin and its receptors in inflammation, adipogenesis, angiogenesis and osteoclastogenesis [13, 14]. Although experimental studies confirmed visceral fat origin of chemerin, clinical studies have not confirmed which obesity indicators, anthropometric or quantitative measurements can show increased secretion of this adipokine. Therefore, the aim of this study was to investigate association between anthropometric indices, ultrasonographic measurement of visceral and subcutaneous fat tissue thickness and levels of certain proinflammatory adipokines (chemerin and resistin).

Methods

Study design and inclusion criteria

In this cross-sectional study, we used laboratory data, anthropometric measurements, as well as ultrasonographic measurements of subcutaneous and visceral fat tissue thickness. The research was conducted at the Clinic for Endocrinology, Diabetes and Metabolic Diseases, in collaboration with the Center for Laboratory Medicine and Center for Radiology, Clinical Center of Vojvodina, Novi Sad, Serbia. The study comprised a consecutive sample of 60 obese patients (BMI $\geq$ 30 kg/m^2), without diabetes mellitus [fasting glucose level $\leq$ 7.0 mmol/L and glycolized hemoglobin A1c (HgbA1c) $\leq$ 42 mmol/L]. Exclusion criteria were: presence of autoimmune, infectious, malignant and psychiatric diseases, along with previously confirmed cardiovascular diseases. Another exclusion factor was changes of body weight within the past three months. The control group comprised 20 respondents with normal body mass (BMI < 25 kg/m^2), clinically healthy, and sex and age matched with obese patients. The study was conducted in accordance with the Helsinki declaration, and approved by Ethics Committee of the Clinical Center of Vojvodina (No 00-15/134). Each examinee signed informed consent. Patients' privacy was guaranteed by coding laboratory samples (each sample was assigned with a unique number).

Study protocol

All respondents were outpatients administered to the Clinic for Endocrinology, Diabetes and Metabolic Diseases in order to analyze specific anthropometric parameters, clinical examination, laboratory analysis and ultrasonographic examination. Before obtaining blood samples, patients were asked to restrain from physical activity and alcohol intake for 24 h. Fasting for 12 h was mandatory before taking blood samples from cubital vein. Standard analyses were performed immediately afterwards, except for serum levels of chemerin and resistin analysis, for which, after centrifugation, samples were frozen at temperature of -20° C no longer than 4 weeks.

Anthropometric measurements

Body height (BH) was measured using a Martin anthropometer with accuracy of 0.1 cm, body mass (BM) was measured with medical decimal scale with accuracy of 0.1 kg. BMI was calculated using formula BM/BH^2 (kg/m^2). Neck circumference (NC), WC and HC were measured by measuring tape, with precision of 0.1 cm. ABSI was calculated using formula: WC x $BM^{-2/3}$ × body $weight^{5/6}$. WHR was calculated using formula WC/HC. Total fat BM (FAT mass, kg) and percentage of fat BM (FAT mass, %) were estimated by bioelectrical impedance analysis (TANITA TFB-310 Body Composition Analyzer; Tanita, Tokyo, Japan).

Laboratory measurements

Plasma glucose level was analyzed using standard method (enzyme UV test), insulin level was analyzed using standard method [Chemiluminescent Microparticle Immuno Assay (CMIA), using device ADVIA Centaur XP, Siemens, USA]. Insulin resistance index [homeostasis model assessment of insulin resistance (HOMA-IR)] was defined by basal concentration of glucose (G) and insulin values, using formula: HOMA-IR = G × I_0/22.5 [15]. Concentrations of total cholesterol (C), triglycerides, HDL-C and LDL-C were analyzed by direct enzyme method (Architect *ci* 4,100 Abbott, USA). Magnesium (Mg^{2+}, mmol/L) level was determined spectrophotometrically on the Mindray biochemical analyzer by applying commercial sets. Serum concentrations of chemerin and resisitin were determined using the Human Chemerin Quantikine ELISA set and the Human Duo Set ELISA set, respectively (R&D Systems, Minneapolis, USA), according to user manual.

Ultrasonographic measurement of visceral and subcutaneous fat tissue thickness was performed on the GE Logiq 7 ultrasonography machine, with high frequency linear probe (12 Hz for subcutaneous fat thickness) and low frequency convex probe (5 MHz for visceral fat thickness) [16].

The parameters of the visceral and subcutaneous fat tissue thickness were obtained by a standard examination; the patients were examined during the expiratory phase of a quiet respiration, and the transducer was applied on the body surface without undue pressure. Minimal subcutaneous fat (Min SFT) was defined as thickness of the fat tissue layer measured as the distance from the skin surface to the *linea alba*, under the xiphoid process The maximum thickness of subcutaneous fat tissue-a (Max SFTa) was defined as distance from the skin surface to the *linea alba*, measured 2 cm above the umbilical cord. The maximum thickness of subcutaneous fat tissue-b (Max SFTb) was defined as the distance from the skin surface to the *linea alba*, measured 2 cm below the umbilical cord. Thickness of intraabdominal fat tissue (IAFT) was defined as the distance between the posterior aspect of the *rectus abdominis* muscle and the anterior aortic wall, measured 2 cm above the umbilical cord. Visceral fat (VF) was defined as the distance between posterior aspect of the *rectus abdominis* muscle and anterior aspect of the paravertebral muscles measured at the umbilical level [16].

Two radiologists, with 15 and 7 years of experience in abdominal ultrasonography, performed double-blinded independent measurements (IAFT, Max SFTa, Max SFTb and VF). Interobserver agreement of the measurements was analyzed in the group of 20 obese patients. The coefficient of variation (intraobserver and interobserver technical error) for IAFT was 3.6% and 4.2%; Max SFTa was 2.7% and 3.6%; Max SFTb was 4.1% and 3.3%; and VF was 4.3% and 6.2%, respectively.

Statistical methods

The Shapiro-Wilk test was used in testing for normality. Data were presented as mean ± standard deviation for normally distributed continuous variables and median (interquartile range) for nonparametric continuous variables, while categorical data were presented as the number of the observations divided with the total number of respondents within the group. Parametric (*t*-test) and nonparametric (Mann-Whitney) statistical tests were used. The χ^2 test was used for categorical variable (gender). Correlations between serum profile of adipokines and tested parameters were evaluated by the Pearson's or Spearman's coefficients. A multivariable regression analysis was used to assess the associations between serum chemerin and resistin levels, and anthropometric characteristic, ultrasonographic measurements of visceral and subcutaneous fat tissue thickness and metabolic biochemical characteristics. Statistical analysis of obtained data was performed using IBM Statistics SPSS 23 package (Chicago, Ilinois, USA).

Results

Table 1 shows the results of anthropometric measurements in the study groups. Obese respondents had significantly higher anthropometric indices (BMI, Fat, Fat Mass, ABSI, NC, WC, HC, WHR) compared to the control group of normally nourished respondents ($p < 0.05$ and $p < 0.01$, respectively).

Descriptive characteristics of visceral and subcutaneous fat tissue thickness measurements by ultrasonography for all respondents are presented in Table 2. A subcutaneous fat tissue thickness measurements (Min SFT, Max SFTa and Max SFTb) were significantly higher in the obese group

Table 1

Anthropometric characteristics of study groups

Variable	Obese group (n = 60)	Control group (n = 20)	*p*
Age (years), mean ± SD	38.7 ± 8.3	37.1 ± 4.7	0.04
Male, n (%)	21 (35.0)	10 (50.0)	0.35
BMI (kg/m^2), median (IQR)	35 (32.00–40.00)	23 (21.00–25.00)	< 0.01
Fat (%), mean ± SD	48.3 ± 10.1	22.6 ± 4.9	< 0.01
Fat Mass (kg), mean ± SD	51.9 ± 14.9	15.8 ± 3.3	< 0.01
ABSI, median (IQR)	0.078 (0.063–0.087)	0.075 (0.083–0.068)	0.02
NC (cm), median (IQR)	39.5 (37–44)	35 (32–37)	< 0.01
WC (cm), mean ± SD	111.8 ± 14.6	80 ± 8.85	< 0.01
HC (cm), median (IQR)	117.5 (110.5–122)	86 (84–94)	< 0.01
WHR, median (IQR)	0.95 (0.9–1.0)	1.1 (0.9–1.1)	0.04

BMI – body mass index; ABSI – A Body Shape Index; NC – neck circumference; WC – waist circumference; HC – hip circumference; WHR – waist to hip ratio; SD – standard deviation; IQR – interquartile range.

Table 2

Measurement of visceral and subcutaneous fat tissue thickness by ultrasonography

Variable	Obese group (n = 60) mean ± SD	Control group (n = 20) mean ± SD	*p*
IAFT (mm)	46.7 ± 28.9	24.2 ± 9.1	< 0.01
Max SFTa (mm)	33.6 ± 17.9	16.9 ± 5.3	< 0.01
Max SFTb (mm)	37.3 ± 19.6	20.2 ± 7.6	< 0.01
Min SFT (mm)	26.7 ± 15.2	17 ± 5.8	< 0.01
VF (mm)	51.1 ± 17.8	13.5 ± 37.2	< 0.01

IAFT – intraabdominal fat tissue thickness; Max SFTa – maximum thickness of the subcutaneous fatty tissue-a; Max SFTb – maximum thickness of the subcutaneous fatty tissue-b; Min SFT – minimal subcutaneous fat tissue thickness; VF – visceral fat tissue thickness; SD – standard deviation.

Table 3

Biochemical characteristics of study groups

Variable	Obese group (n = 60)	Control group (n = 20)	*p*
FPG (mmol/L), median (IQR)	5.2 (4.8–5.6)	4.9 (4.6–5.2)	< 0.01
HDL–C (mmol/L), mean ± SD	1.2 ± 0.3	1.5 ± 0.3	< 0.01
Triglycerides (mmol/L), median (IQR)	1.4 (1.1–2)	0.9 (0.5–1.2)	< 0.01
LDL–C (mmol/L), median (IQR)	3.2 (2.6–3.9)	2.6 (2.2–3.6)	= 0.04
FPI (mU/mL), median (IQR)	13.4 (10.4–18.6)	4.9 (4–6.1)	< 0.01
HOMA–IR, median (IQR)	2.4 (1.9–3.9)	0.7 (0.6–0.8)	< 0.01
Chemerin (ng/mL), median (IQR)	41.6 (26.2–61.1)	18.3 (14.5–26.7)	< 0.01
Resistin (ng/mL), median (IQR)	4.72 (4.27–5)	4.0 (2.9–4.7)	< 0.01
Mg^{2+} (mmol/L), mean ± SD	0.83 ± 0.07	0.82 ± 0.06	= 0.46

FPG – fasting plasma glucose; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; FPI – fasting plasma insulin; HOMA-IR – homeostasis model assessment of insulin resistance; SD – standard deviation; IQR – interquartile range.

compared to the control one ($p < 0.01$, for all). Also, the obese respondents had significantly higher VF compared to the control ones ($p < 0.01$).

All metabolic biochemical parameters (Table 3) were significantly higher in the obese compared to the control group ($p < 0.05$ and $p < 0.01$, respectively).

Results of correlation analysis between serum profile of adipokines and tested parameters are shown in Table 4. The serum levels of chemerin and resistin correlated positively with BMI, FAT (%), FAT (kg), VF and HOMA-IR ($p < 0.05$, for all). Also, we observed significant correlation between resistin and NC ($r = 0.23$, $p = 0.03$) and ABSI ($r = 0.22$, $p = 0.04$).

Chemerin levels correlated positively with FPI ($r = 0.45$, $p = 0.01$) and tryglicerides ($r = 0.36$, $p = 0.01$). Negative correlation was observed between resistin and HDL-C ($r = -0.31$, $p = 0.03$).

In multivariable linear regression analysis, chemerin ($\beta = 0.23$; $p = 0.008$) and resistin ($\beta = 0.43$; $p = 0.002$) levels were independently and significantly associated only with VF.

Discussion

Obesity is a disease in which individuals with the same or similar BMI can develop different metabolic, cardiovascular diseases, and even tumors. However, BMI

Table 4

Correlation between adipokines and anthropometric and biochemical characteristics and ultrasonographic measurements in all respondents

Parameter	Chemerin (ng/mL)		Resistin (ng/mL)	
	r	*p*	*r*	*p*
BMI (kg/m^2)	0.42	0.00	0.26	0.017
WC (cm)	0.31	0.07	0.29	0.07
HC (cm)	0.29	0.01	0.25	0.23
NC (cm)	0.15	0.19	0.23	0.03
ABSI	0.13	0.26	0.22	0.04
FAT (%)	0.36	0.01	0.26	0.019
FAT mass (kg)	0.3	0.01	0.25	0.02
VF (mm)	0.49	0.00	0.48	0.00
Min SFT (mm)	0.112	0.322	-0.045	0.677
Max SFTa (mm)	0.103	0.364	-0.023	0.829
Max SFTb (mm)	0.165	0.143	-0.089	0.413
IAFT (mm)	0.108	0.342	-0.033	0.763
Max PFT (mm)	0.173	0.125	-0.092	0.399
FPG (mmol/L)	0.067	0.555	0.019	0.859
FPI (mU/mL)	0.45	0.001	0.272	0.011
HOMA-IR	0.368	0.01	0.316	0.05
Triglycerides (mmol/L)	0.365	0.01	0.195	0.07
HDL-C (mmol/L)	0.212	0.058	-0.317	0.03
LDL-C (mmol/L)	0.139	0.219	0.163	0.132

BMI – body mass index; ABSI – a body shape index; NC – neck circumference; WC – waist circumference; HC – hip circumference; WHR – waist to hip ratio; IAFT – intraabdominal fat tissue thickness; Max PFT – maximum preoperational fatty tissue thickness; Max SFTa – maximum thickness of the subcutaneous fatty tissue-a; Max SFTb – maximum thickness of the subcutaneous fatty tissue-b; Min SFT – minimal subcutaneous fat tissue thickness; VF – visceral fat tissue thickness; FPG – fasting plasma glucose; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; FPI – fasting plasma insulin HOMA-IR – homeostasis model assessment of insulin resistance; *r* – coefficient of correlation.

does not take into account fat tissue compartments [16]. Indices as WC, HC and WHR are relatively simple, reproducible method which can show fat tissue distribution, but their limitation is inability to precisely assess the amount of fat tissue. Bioelectrical impedance analysis is a method which can assess precise distribution, percentage and amount of fat BM in a total BM, and differ fat from non-fat BM [17]. NC and ABSI are new parameters, whose practical value has not been sufficiently examined yet. Ultrasonographic measurement of fat tissue thickness is available, cheap and reproducible [18], with capability of analyzing subcutaneous and visceral fat depot, and has advantage in assessment of complications related to obesity. To assess functional characteristics of the fat tissue, serum concentrations of proinflammatory adipokines, chemerin and resistin are measured.

In our study, obese patients had significantly higher values of BMI, FAT mass (kg), percentage of fat adipose tissue (FAT, %) and WC. Also, we proved statistically significantly higher values of NC in the group of obese patients compared to the group with normal nutritional status. NC correlates with metabolic disorders, such as hypertriglyceridemia, insulin resistance, type 2 diabetes mellitus etc. Moreover, it has been proven that NC above 37 cm in men and 34 cm in women are reliable indicators for presence of abdominal obesity [19]. This study showed statistically positive correlation between NC and resistin level in the group of obese respondents, which has been confirmed in only one study [20]. Resistin is proinflammatory adipokine, and correlation between resistin and NC can point out high risk obesity in examined group, with impact on different organ systems.

Along with NC, ABSI also had statistically higher values in the obese group in comparison to the control one. ABSI is a good anthropometric parameter for defining comorbidities related to obesity, chronic diseases and lethal outcome [21–23]. In our study, ABSI also had statistically significant correlation with resistin levels. This finding is even more significant considering that resistin is proinflammatory adipokine, and that its elevated concentrations in obese patients can indicate increased risk for numerous diseases related to obesity. To our knowledge, review of the literature showed no information on relation between resistin and ABSI.

In our research, obese patients had statistically significantly higher values of the subcutaneous and VF tissue compartment thickness compared to those in the control group. Also, the VF tissue thickness had statistically significant correlation with serum concentrations of chemerin and resisitin. From pathophysiological aspect, this finding is very important considering that chemerin is adipokine with numerous different roles in human body, and that chemerin expression is higher in the visceral than in subcutaneous fat tissue [24]. These findings indicate that intrinsic factors of the VF tissue can play a crucial role in understanding main pathophysiological mechanisms which

lead to development of cardiovascular diseases, metabolic syndrome and inflammation in visceral adiposopathy [25]. Study of Schlecht et al. [26] did not prove statistically significant correlation between resistin values and the VF tissue measured by ultrasound, while research of McTernan et al. [27], Azuma et al. [28] and Jain et al. [29] showed positive correlation of the VF tissue amount and serum values of proinflammatory resistin. Such controversial results imply the necessity of additional researches to further explain the connection of resistin and adipose tissue.

Beside anthropometric parameters and fat tissue thickness measurements, metabolic characteristics of the obese respondents showed significantly higher values of glycemia, insulinemia, HOMA-IR and proatherogenic cholesterol (LDL-C) and triglycerides, together with statistically significantly lower values of HDL-C compared to respondents with normal nutritional status. Moreover, there was statistically positive correlation between chemerin and insulin, HOMA-IR and triglycerides levels, as well as statistically positive correlation of resistin and HOMA-IR, and negative correlation of resistin and HDL-C. Multicentric study of American authors [30] also showed positive correlation of chemerin and values of insulin, HOMA-IR and triglycerides in patients with newly discovered metabolic syndrome. The same study proved positive correlation of chemerin and the subcutaneous fat tissue, which was not confirmed in our study. Research of German authors [31] showed that chemerin is a good biomarker of insulin resistance in healthy adult men. However, data regarding dyslipidemia and inflammation are yet debatable [32, 33]. Due to this, further research of chemerin role in metabolic disorders is highly desirable.

It is known that resistin is secreted from adipocytes and macrophages, and is also a trigger for development of insulin resistance. In most of the published studies, increased resistin values were detected in obesity, metabolic syndrome, and type 2 diabetes mellitus [34–36]. Our study did not confirm statistically positive correlation between resistin and triglycerides levels as parameter of metabolic syndrome. Studies of de Luis et al. [37], and Uslu et al. [38] showed positive correlation of resistin with LDL-C, and negative correlation with HDL-C fraction, which is in compliance with results of our study. Hypothesis of inverse correlation of resistin with cholesterol serum levels can be explained by resistin dependent cholesterol sequestration in macrophages. Another possible explanation of inverse correlation between resistin and HDL-C can be explained by the fact that cholesterol concentration depends on resistin concentration [39–41]. Our study proved statistically positive correlation between resistin and HOMA-IR, which was confirmed in previous studies [42].

Advantage of our study was direct comparison of two actual adipocytokines with precisely defined amount of the fat tissue in specific compartments and with anthropometric parameters, as well as correlation of proinflammatory adipokines with metabolic markers. The main limitations of the current study were small sample size and cross-sectional study design. Also, as the measurement of serum concentration of chemerin and resistin is not current practice, there is a limited implementation of this study in general practice.

Therefore, further prospective studies could have far more significant role in defining and monitoring comorbidities related to obesity. Early identification of pathogenetic factors in development of obesity related comorbidities (insulin resistance, chronic inflammation, fat tissue dysfunction etc.) could result in personalized prevention and/or personalized treatment of obese, high-risk patients.

Conclusion

There is positive, statistically significant correlation between obesity indices, both classical and newer ones, and the level of proinflammatory cytokines (chemerin and resistin). Ultrasonographically measured VF thickness may improve assessment of proinflammatory fat tissue characteristics. Further studies are needed to precisely define the use of ultrasonographic fat tissue measurements in clinical practice.

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Non-melanoma skin cancers in Serbia (1999–2015) – the need for national prevention and control strategy

Nemelanomski karcinomi kože u Srbiji (1999–2015) – potreba za nacionalnom strategijom za prevenciju i kontrolu

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Abstract

Background/Aim. Non-melanoma skin cancers (NMSC) are ones of the most rapidly increasing cancers worldwide. Although NMSCs have a relatively low mortality rate, they are an important public health concern and the most costly cancers in many countries. The two main objectives in this study were: first, to analyze the trend of age-standardized incidence rate of NMSCs in Serbia and second, to assess the need for national prevention and control strategy based on analyzed trend. **Methods.** From the Serbian Cancer Registry, we extracted all cases of NMSCs registered in central Serbia from January 1, 1999 to December 31, 2015. Joinpoint regression analysis was used to define trends and annual percentage change (APC). **Results.** NMSCs significantly increased for both genders with APC of +2.32% ($p < 0.001$). Significantly increasing trend of incidence rates was higher in women (APC, +2.63%; $p < 0.0001$) than in men (APC, +2.01%; $p < 0.001$). **Conclusion.** Our results show a continuously increasing incidence rate of NMCS in Serbia. Without the national preventive strategy, current sporadic activities are highly unlikely to result in reducing the growing trends.

Key words:
skin neoplasms; carcinoma, basal cell; carcinoma, squamous cell; incidence; serbia.

Apstrakt

Uvod/Cilj. Nemelanomski karcinomi kože (engl. *non-melanoma skin cancers* – NMSC) su jedni od najbrže rastućih karcinoma širom sveta. Iako imaju relativno nisku stopu smrtnosti, zbog cene koja je potrebna za njihovo lečenje, predstavljaju veliku brigu u javnom zdravlju mnogih država. Ova studija imala je dva osnovna cilja: prvi, da analizira trend standardizovane incidencije NMCS u Srbiji i drugi, da se na osnovu anliziranog trenda proceni potreba za izradu nacionalne strategije za prevenciju i kontrolu ovog karcinoma. **Metode.** Iz Registra za rak Srbije izdvojeni su svi slučajeve NMCS registrovani u centralnoj Srbiji od 1. januara 1999 do 3. decembra 2015. godine. Trend kretanja i godišnji procenat promene incidencije (engl. *annual percentage change* –APC) izračunat je regresionom analizom pomoću tačke spajanja. **Rezultati.** NMCS su se značajno povećavali kod oba pola sa APC od +2,32% ($p < 0,001$). Značajno povećanje trenda incidencije je bilo veće kod žena (APC, +2.63%; $p < 0,0001$) nego kod muškaraca (APC, +2.01%; $p < 0,001$). **Zaključak.** Rezultati pokazuju kontinuirano povećanje stope incidencije NMCS u Srbiji. Bez nacionalne preventivne strategije vrlo je malo verovatno da će sadašnje sporadične preventivne aktivnosti smanjiti ovaj rastući trend.

Ključne reči:
koža, neoplazme, karcinom, bazocelularni; karcinom, planocelularni; incidenca; srbija.

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Introduction

Non-melanoma skin cancers (NMSC) are the most common cancers in the world. Although these cancers include other rare cutaneous neoplasms, the term generally refers to basal cell carcinoma (BCC) and squamous cell skin carcinoma (SCC) [1]. NMSC are ones of the most rapidly increasing cancers worldwide [2, 3]. The world's highest incidence is among the white population of Australia and New Zealand [4] and it is very low among the black population [5]. In the United States NMSC account for over 5.4 million cases in more than 3.3 million people and more people have had skin cancer than all other cancers combined [6].

The primary risk factor for developing NMSC is exposure to ultraviolet radiation (UVR). UVR induced deoxyribonucleic acid (DNA) damage causes genetic alterations which play a crucial role in skin photoaging and the genesis of skin cancer [7, 8]. Some studies are shown that outdoors working individuals have 40% to 80% higher risk for developing BCC and SCC due to exposure to sunlight [9, 10]. Indoor tanning is also highly associated with an increased risk of both NMSC and the risk is higher with use in early life [11, 12]. Other risk factors include the phenotype of an individual characteristics such as fair skin, blue eyes and red hair [13]. Although NMSC have a relatively low mortality rate, they are an important public health concern and the most costly cancers in many country [14–17].

Collecting data on NMSC incidence rate based on national cancer registers is important because they provide information for planning health policies and assist in understanding needs and effectiveness of prevention for that particular region [17, 18].

Skin cancer prevention activities have been performed throughout the entire world. In Europe, Euromelanoma as a pan-European prevention programme against NMSC and melanoma started in 1999 and spread in 33 countries (Serbia included) [19–21]. However, public health significance of NMSC seems to be unrecognized in Serbia, there is no national strategy for prevention and control of these cancers and only a few studies reported incidence rate of NMSC in Serbia [18, 22].

There were two main objectives of this study: first, to analyze the trend of age standardized incidence rate of NMCS in Serbia and second, to assess the need for national prevention and control strategy based on analyzed trend.

Methods

Type of the study and data sources

In this retrospective descriptive epidemiological study, the incidence rate of NMSC in Serbia during a 17-year study period (1999–2015) was estimated. The Serbian Cancer Registry (the Registry) had been established in 1970 and became a member of the International Agency for Research on Cancer (IACR) and European Network of Cancer Registries (ENCR) in 1998. All health institutions in Serbia (private and state) are mandated by law to submit a report on all new cases of malignant tumours to The Registry. Our study was conducted in central Serbia (excluding provinces Vojvodina and Kosovo and Metohija) which had a population of 5,506,936 in 1999 and 5,203,682 in 2015. Information on the size and migration population in the past was provided by the Statistical Office of the Republic of Serbia.

Statistical analysis

From The Registry, we extracted all cases of NMSC registered in central Serbia from January 1, 1999 to December 31, 2015 based on the International Classification of Diseases, Tenth Revision (ICD-10) code C44 [23]. Age-specific incidence rates were calculated using the following age groups: below 39, 40–49, 50–59, 60–69 and over 70 years of age. To allow comparison between our data and data from other regions, age-standardized incidence rates (ASIR) were calculated using the direct standardization method to the world population [24]. Incidence rates were reported as the incidence per 100,000 persons yearly. Trends and annual percentage change (APC) of the incidence rate with corresponding 95% confidence intervals (CI) were calculated by performing joinpoint regression analyses using the Joinpoint Regression Software version 4.6.0.0 (available at https://surveillance.cancer.gov/joinpoint/download). The trend was considered as significant when the *p*-value was below 0.05 ($p < 0.05$).

Results

During the 17-years period (from January 1,1999 to December 31, 2015) a total number of 48.488 persons (25.213 men and 23.275 women) with primary non-melanoma skin cancer were reported to the National Cancer Registry of Central Serbia. Women represented 48% and men 52% of all persons. Table 1 shows age-specific incidence rates, crude rate, and age-standardized incidence rate (world standard population) per 100.000 persons for all, men and women. The age-specific incidence rates were the highest in the age group over 70 years and approximately 50% (49.9% male and 50.8% female) of all registered cases were in this age group. About 3% (2.3% male and 3.2% female) of all registered cases of NMSC belonged to a group under 39 years of age. Table 2 presents world age-standardized rate and number of patients with primary NMSC in Serbia through the entire study period.

The lowest level of ASIR of NMSC in the Republic of Serbia was in the first year of the observation period, in 1999 (19.74 per 100.000 men, 95% CI: 18.46–21.01 and 15.22 per 100.000 women). ASIR is gradually increasing and reached the highest level in 2014 (33.31 per 100.000 men, 95% CI: 31.78–34.84 and 26.12 per 100.000 women, 95% CI: 24.89–27.36). Based on the joinpoint analysis, ASIR of NMSC in Serbia significantly increased, for both gender combined, in the period 1999–2015 with APC of +2.32% (95% CI: 1.60–3.10, $p < 0.001$) (Figure 1).

Table 1

Age-specific, age-standardized incidence rate (per 100.000 persons), crude rate and number of patients with primary non-melanoma skin cancer in Serbia, 1999–2015

Parameters	Male		Female		Overall	
	n	I	n	I	n	I
Age group (years)						
≥ 39	578	19.16	739	24.56	1317	21.85
40–49	1280	40.20	1212	37.14	2492	38.65
50–59	3636	116.18	3208	96.62	6844	106.10
60–69	7148	286.82	6060	210.45	13208	245.84
≤ 70	12571	549.32	12056	373.25	24627	446.46
Crude rate*	N/A	56.70	N/A	49.64	N/A	53.08
WASR*	N/A	29.53	N/A	22.76	N/A	25.74

n – number of patients; I – incidence; N/A – not applicable; WASR – World age-standardized rate; *all ages.

Table 2

Age-standardized incidence rate (per 100.000 persons) and number of patients with primary non-melanoma skin cancer in Serbia, 1999–2015

Year	Males			Females			Overall		
	n	WASR	95% CI	n	WASR	95 % CI	n	WASR	95 % CI
1999	916	19.74	18.46–21.01	843	15.22	14.20–16.25	1759	17.21	16.40–18.01
2000	1227	26.62	25.13–28.10	1066	19.46	18.29–20.63	2293	22.62	21.70–23.55
2001	1203	25.67	24.22–27.12	991	17.36	16.28–18.44	2194	21.11	20.23–22.00
2002	1258	26.63	25.16–28.10	1052	18.52	17.41–19.64	2310	22.16	21.25–23.06
2003	1316	27.22	25.75–28.69	1138	19.75	18.61–20.90	2454	23.05	22.14–23.96
2004	1408	29.76	28.21–31.32	1365	24.13	22.85–25.41	2773	26.62	25.63–27.61
2005	1370	27.56	26.10–29.02	1269	21.88	20.67–23.08	2639	24.31	23.38–25.24
2006	1731	35.33	33.66–36.99	1633	27.73	26.39–29.08	3364	31.07	30.02–32.12
2007	1472	29.53	28.02–31.03	1356	22.70	21.49–23.91	2828	25.72	24.78–26.67
2008	1644	32.33	30.76–33.89	1577	25.40	24.14–26.65	3221	28.44	27.46–29.43
2009	1679	33.53	31.93–35.14	1506	25.21	23.94–26.49	3185	28.91	27.91–29.92
2010	1569	30.83	29.31–32.36	1531	25.25	23.99–26.52	3100	27.69	26.72–28.67
2011	1593	30.90	29.38–32.42	1506	24.72	23.47–25.96	3099	27.41	26.45–28.38
2012	1622	30.73	29.24–32.23	1507	24.15	22.93–25.37	3129	27.06	26.11–28.00
2013	1681	30.69	29.23–32.16	1689	26.04	24.80–27.29	3370	28.00	27.06–28.95
2014	1830	33.31	31.78–34.84	1717	26.12	24.89–27.36	3547	29.27	28.31–30.23
2015	1694	31.44	29.94–32.94	1529	23.40	22.23–24.58	3223	26.98	26.04–27.91

n – number of patients; WASR – world age-standardized rate; CI – confidence interval.

ASIR was higher in women Significantly increasing trend of (APC, +2.63%; 95% CI: 1.50–3.80; $p < 0.0001$) (Figure 2) than in men (+APC, 2.01%; 95% CI: 1.01–3.10; $p < 0.001$) (Figure 3).

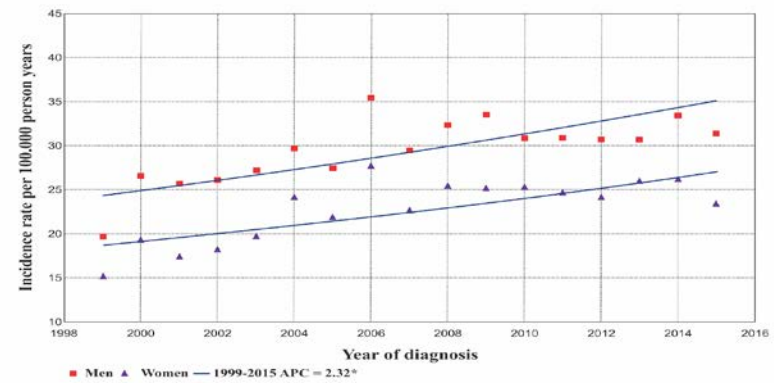

Fig. 1 – Joinpoint analyses of age standardized incidence rates (world standard population) of non-melanoma skin cancer in Serbia, 1999–2015, men and women combined, with annual percentage change (APC).

***significant increase.**

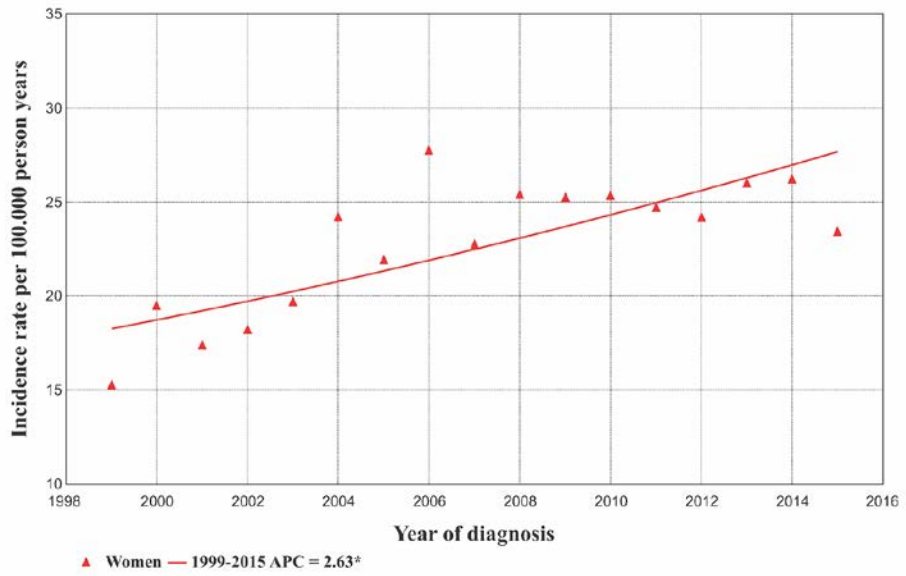

Fig. 2 – Joinpoint analyses of age standardized incidence rates (world standard population) of non-melanoma skin cancer in Serbia, 1999–2015, with annual percentage change (APC) for women. *significant increase.

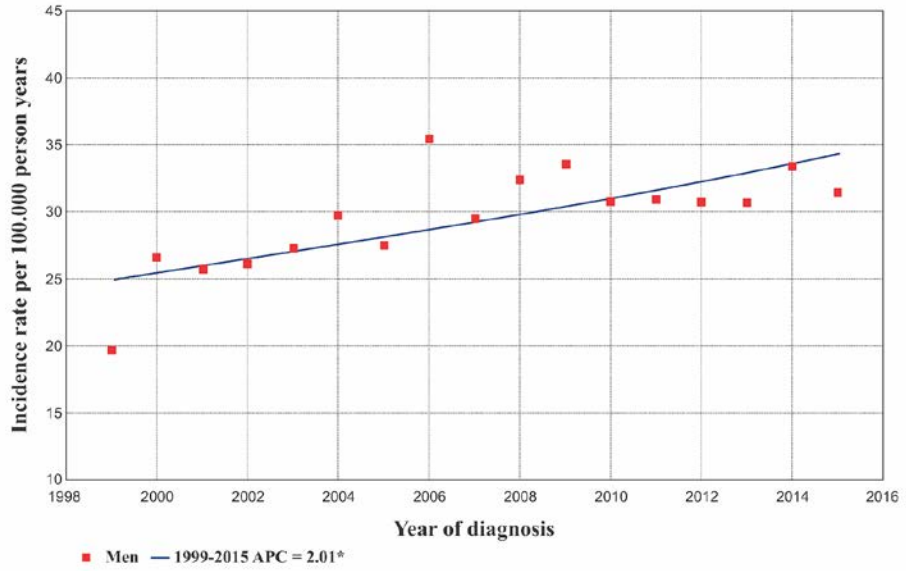

Fig. 3 – Joinpoint analyses of age standardized incidence rates (world standard population) of non-melanoma skin cancer in Serbia, 1999–2015,with annual percentage change (APC), for men. *significant increase.

Discussion

This national register-based study analyzed trends of NMSC over a period of 17 years. It it is one of the few studies [18, 22] describing the incidence of NMSC in the Republic of Serbia. The study showed that NMSC are the most common among the elderly; the age-specific incidence rates is the highest in the age group over 70 years (50%) and very low in the younger age (3%). The age distribution of NMSC cases was similar to other reports [4, 25–28]. Many studies have confirmed that cumulative exposure to UVR, especially in the first two decades of life, plays the key role in carcinogenic process of skin cancers [7, 8]. The reason for the highest age-specific incidence rates in elderly in our study is probably the ageing of the population in Serbia. Another reason for prevalence of skin cancers in the age group over 70 years could be a lack of information regarding the importance of harmfulness of excessive exposure to UVR among the elderly population at the time in their youth. Although the incidence rate is very low in younger age this is the right time to start with preventive activities. NMSC generally take many years to appear but are usually caused during young age. The most of the damage resulting in skin cancers occurs in the adolescent and childhood and therefore prevention strategies need to focus on sun protection

education especially towards these age groups [29, 17]. Thus, schools are the most important institution where awareness of NMSC and education of sun protection should start. In Australia, the country with the highest incidence rate of NMSC in the world, SunSmart Schools Program is the main skin cancer control strategy for more than 20 years. This program includes promoting the use of sun-protective clothing and shade-seeking behavior [30]. Many Australian schools have already improved sun-protective behaviors [31]. In Serbia, there are no national educational school programs focused on sun protection so far. In fact, only one published educational program was implemented for some high school students in Belgrade from 2007 to 2008. The goal of this program was to increase awareness among students of harmfulness of excessive exposure to UVR and to educate them on sun protection measures [32].

After analyzing ASIR of NMSC in our study, noticeable differences between gender were found: they were much higher in men than in women during the whole study period (1999–2015). Our results are compatible with findings from studies in other countries [4, 33–35]. Incidence rates tend to be higher in men than in women most likely because in Serbia, men are more likely to have an outdoor occupation. Another reason could be different way of dressing and hairstyle between the genders.

Age-standardized incidence rates of NMSC in Serbia have increased more than 1.5-fold from 1999 to 2015 and our results showed a continuously increasing incidence rate. The continuously increasing incidence rates of NMSC were also recorded in Australia [4], New Zeland [36], the United States [6], Canada [37], Asia [38, 39] and in European countries: Netherlands [40], Denmark [41], Italy [42] and Switzerland [43]. Explanations for this increasing incidence rates could be several factors. First, and the most likely factor is the increased number of people who are expose to greater UVR compared to prior generations. Spending the holiday at sunny destinations and outdoor sport activities has become more popular and more achievable than in the past, which prolonged exposure to sunshine and increased cumulative ultraviolet exposure consequently. Cumulative ultraviolet exposure is well known risk in photoaging of the skin and genesis of skin cancer [7, 8]. The increasing incidence rate of NMSC in our research may also suggest that people in Serbia are not fully aware of the dangers of the excessive exposure to UVR and that little attention has been paid to raise awareness of the harmful effects of the sun in the past. Euromelanom Serbia, a part of the pan-European association of Euromelanoma Europe, has been implementing significant awareness-raising activities and changing the public's behavior regarding sun protection in recent years. Media campaign of Euromelanoma Serbia under the slogan "Serbia has a skin cancer problem" informs the public about adequate protection against UVR, both from sun and from artificial sources of UVR. This campaign uses various means of public communication to promote skin awareness (brochures, pamphlets, posters, mass media advertising) and benefits from an Internet platform for Serbia (https://www.euromelanoma.org/serbia). Another possible reason for growing skin cancer incidence is depletion of the ozone layer in the atmosphere and the increased air pollution in the past. Depletion of the ozone layer facilitated UVR to reach the Earth's surface and increased the intensity of ultraviolet light exposure [44].

A more significant incidence rate increase of NMSC for women (APC = +2.63%; $p < 0.0001$) compared to men (APC = +2.01%; $p < 0.001$), in our study, is similar to that of the Leiter et al. [34] study from two federal states in Germany. They reported a more significant increase for women than for men between 1999 and 2012 in Schleswig-Holstein federal state (women APC = +3.3%; men APC = +2.3%) and between 1970 and 2012 in Saarland federal state (women APC = +6.3%; men APC = +6.0%). Higher increase in ASIR of NMSC in women was observed also in The Netherlands [45] and Denmark [41]. Gender difference in increasing standardized incidence rate of NMSC can be caused by the use of tanning beds, because women are more prone to use sun tanning or indoor tanning than men. Use of tanning beds started in 1970s for cosmetic use [46] and more than 30 years later Bataille et al. [47], in an epidemiological study on sunbed use in Europe, reported that more than half of Northern European population between 18 and 50 years were using tanning beds. Some other studies have shown that indoor tanning increases the risk of NMSC by 40% to 102% [11], doubling the risk of SCC and increasing risk of BBC by 50% [48]. Since indoor tanning had been identified as carcinogenic, many European countries (France, Spain, Portugal, Germany, Austria, Belgium, England, Wales, Northern Ireland and Scotland) made age restrictions and demanded parental permission for tanning beds use [46]. Brazil outlawed indoor tanning for all age groups since 2011 [46]. There is still no law in Serbia limiting the use of salon tanning and it seems that people are not aware of the dangers of it – in fact many people still believe that tanned skin is more attractive and "looks healthy". Restrictions on the use of salon tanning in Serbia could certainly prevent the growth of incidence rate of NMSC in the future.

At first glance, low mortality rate of NMSC along with the lower age-standardized incidence rates in Serbia (Table 2) than in other countries [4, 6, 42, 43] may not qualify NMSC prevention as the national financial resource's optimal candidate. This attitude is, of course, understandable due to the fact that Serbia is among the poorer countries in the world and already has very high mortality rates in other malignant diseases. However, the growing trend in NMSC incidence rates, shown in our research (Figures 1–3) suggests that this type of carcinoma will undoubtedly become a significant health problem unless prevention measures are timely taken. Without the national preventive strategy, current sporadic preventive activities in Serbia are highly unlikely to result in reducing the growing trends.

Furthermore, we believe that shown NMSC incidence rate in Serbia is lower than the actual one. The reason is that the Serbian Cancer Register, following the recommendations of the IARC and ENCR, records only one NMSC per person, the first one. If a patient has multiple or a reccurent skin cancer – this is recorded as a single case. This method of

recording data could result in underestimation of the true incidence of NMSC in Serbia. In Australia, Keim et al. [49] found that 16% to 56% of patients with primary NMSC developed additional SCC or BCC. A similar underestimation of the true incidence of NMSC has been reported in the United Kingdom [50] and Germany [51].

We strongly hope that our results prove the need to form the national prevention strategy to promote NMSC awareness, provide sun protection education and restrict indoor tanning.

Conclusion

Our results showed a continuously increasing incidence rate of non-melanoma skin cancer in Serbia between 1999 and 2015. We observed much higher age-standardized incidence rates in men than in women but the increase was more significant for women compared to men. Increasing incidence rate of non-melanoma skin cancer could suggest that people in Serbia are not fully aware of the dangers of exposure to ultraviolet radiation and that little attention has been paid in the past to raise awareness of the harmful effects of both sun and indoor tanning. The development of a national prevention strategy that would include raising public awareness of non-melanoma skin cancer, education on sun protection and limitation of the use of sunbed tanning by the law could certainly reduce the morbidity of these cancer in Serbia.

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Significance of hysteroscopy in diagnosis and treatment of congenital uterine anomalies

Značaj histeroskopije u dijagnostici i tretmanu urođenih anomalija materične šupljine

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Abstract

Background/Aim. Hysteroscopy is one of the important methods in the endoscopic diagnosis and treatment of infertility, particularly at the examination of the morphology and function of the uterus. Uterine factor is present at 10% of infertile women, in which changes of the uterus can be congenital or acquired. The aim of this study was to estimate the significance of hysteroscopy in the diagnosis and treatment of congenital anomalies of the uterine cavity in patients in whom there was a reasonable suspicion for them based on prior clinical, ultrasound and hysterosalpingography (HSG) findings. The significance of hysteroscopy is considered in relation to the number of pregnancies achieved depending on the malformations in the 12 months period after the completion of the diagnostic or operational hysteroscopy. **Methods.** The study included 176 patients with congenital uterine anomalies, which had undergone hysteroscopy, diagnostic or operative, in the period from January 1, 2013 till January 1, 2016. Percentage of pregnancy was followed at all patients during the first 12 months after surgery. Patients were divided into two groups: the first group contained women who had pregnancy, and the second group included women who earlier were not pregnant. Their characteristics were compared, such as: age, duration of infertility, previous pregnancy and/or pregnancy loss, HSG and ultrasound findings, types of congenital anomalies, type of hysteroscopy, as well as the existence of associated pathology. **Results.** The mean age of patients tested in the study was 35 years. The group of patients with primary infertility had a total of 107 patients, while 69 patients were with secondary infertility. The average duration of infertility was 3 years in the studied patients. In the 12-months period, 39 of the examined women began pregnancy, which was completed with term delivery in 33 women. χ^2 test showed a statistical significance difference ($p < 0.05$) between the groups of the patients with or without pregnancy after hysteroscopy in relation to infertility types as well as in relation to the number of previous miscarriages. **Conclusion.** *Subseptus* and *septus uteri* were the most common congenital uterine malformations in our patients. Very rare were *uterus arquatus*, *uterus unicornis* and *uterus bicornis*. In our study, 1/5 of examined women achieved pregnancy after hysteroscopy in the reporting period of 12 months, while the majority of these pregnancies ended with term delivery. The percentage of miscarriages in the examined women was reduced from 38% to 15% after hysteroscopy. In women who achieved pregnancy, uterine septum and subseptum were mostly diagnosed and in these patients hysteroscopic resection was successfully performed.

Key words:
hysteroscopy; uterus; congenital abnormalities; diagnosis; gynecologic surgical procedures; pregnancy.

Apstrakt

Uvod/Cilj. Histeroskopija predstavlja jednu od važnih endoskopskih metoda u dijagnostici i lečenju infertiliteta, posebno u ispitivanju morfologije i funkcionalnosti materice. Uterusni faktor je zastupljen kod oko 10% infertilnih žena, pri čemu promene u materici mogu biti urođene i stečene. Cilj rada je bio da se proceni značaj histeroskopije u dijagnostici i tretmanu urođenih anomalija materične šupljine kod osoba ženskog pola kod kojih je postojala osnovana sumnja za to na osnovu prethodnog kliničkog, ultrazvučnog i histerosalpingografskog (HSG) pregleda. Značaj histeroskopije je posmatran u odnosu na broj ostvarenih trudnoća u zavisnosti od dijagnostikovane anomalije u periodu od 12 meseci nakon obavljene histeroskopije u dijagnostičke ili operativne svrhe. **Metod.**

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Studijom preseka je bilo obuhvaćeno 176 ispitanica sa kongenitalnim anomalijama materice kod kojih je urađena histeroskopija, dijagnostička ili operativna, u periodu od 1.1.2013–1.1.2016. godine. Kod svih ispitanice je praćen procenat nastalih trudnoća u toku prvih 12 meseci posle operacije. Na taj način formirane su dve grupe ispitanica: one koje su ostvarile trudnoću i one koje nisu, i njihove karakteristike su upoređivane: godine života, trajanje infertiliteta, prethodne trudnoće, ultrazvučni i HSG nalazi, vrste kongenitalnih anomalija, vrste histeroskopskog tretmana, kao i postojanje udružene patologije. **Rezultati. S**rednja životna dob ispitivanih žena iznosila je 35 godina. Grupu sa primarnim infertilitetom činilo je ukupno 107 žena, dok je u drugoj grupi (sa sekundarnim infertilitetom) bilo 69 žena. Prosečna dužina trajanja infertiliteta kod ispitivanih osoba iznosila je 3 godine. U periodu od 12 meseci posle operacije, kod 39 žena je došlo do trudnoće, koja je kod njih 33 završena porođajem u terminu. χ^2 testom je dobijena statistički značajna razlika ($p < 0.05$) između posmatranih grupa (sa i bez ostvarene trudnoće posle histeroskopije) u odnosu na vrstu infertiliteta, kao i broj prethodnih pobačaja. **Zaključak.** Od svih kongenitalnih anomalija materice, najzastupljeniji su bili *uterus subseptus* i *septus*, a ređe zastupljeni su bili *uterus arquatus*, *uterus unicornis* i *uterus bicornis*. U našem istraživanju, jedna petina ispitanica je ostvarila trudnoću nakon histeroskopije u posmatranom periodu od 12 meseci i kod većine njih trudnoća je bila završena porođajem u terminu. Procenat pobačaja kod ispitivanih žena bio je smanjen sa 38%, pre histeroskopije, na 15%, nakon histeroskopije. Kod žena koje su ostvarile trudnoću, većinom su dijagnostikovani septum i supseptum uterusa i kod njih je sa uspehom je izvršena histeroskopska resekcija promena.

Ključne reči:
histeroskopija; materica; anomalije; dijagnoza; hirurgija, ginekološka, procedure; trudnoća.

Introduction

The absence of pregnancy during one year of marital life and regular sexual relations, without the use of contraceptive means, is marked as infertility and represents a reversible condition.

During intrauterine development and differentiation of organs, disorders as congenital malformations of the genital organs can occur. Their frequency, according to literature data, is 0.5%–1% in the general population of women. The uterine factor is present at approximately 10% of infertile women.

In female embryos, mesonephric channels caese to exist, while paramezonephric channels evolve, merging in the central part. The cranial horizontal parts of the Miller canal remain unbroken and evolve into the Falopian tube, while the middle and caudal parts merge and form an uterovaginal channel, from which the upper third of the vagina and the epithelium of the body and neck of the uterus are developed. Stroma of the uterus and myometrium originate from the splanchic mesoderm. The Miller tuberculum of the urogenital sinus thickens and forms two parts of the vaginal plate, which separate from it with the lumen that forms the lower two thirds of the vagina.

The occurrence of complete and incomplete longitudinal septum happens in 30–35%, which makes it most common among uterine anomalies. The septum is represented by poor vascular fibro muscular tissue and leads to spontaneous abortion. A complete longitudinal septum extends from the fundus to the inner cervix, dividing the cavum into two parts; it is commonly associated with the longitudinal septum in the vagina. The incomplete septum does not reach the inner cervix and there is communication between the parts of the cavity. During the first trimester, the risk of spontaneous abortion in patients with uterine septum is elevated, and the cause is a poorer vascularization of the implantation site.

The aims of this study were to: 1) evaluate the importance of hysteroscopy in the diagnosis and treatment of congenital anomalies of the uterine cavity in patients with diagnosed infertility; 2) determine the types and frequency of congenital anomalies in the investigated groups of patients; 3) estimate the prevalence of previous pregnancies and their outcome in patients with diagnosed anomalies; 4) determine type of hysteroscopy treatment and its safety (existence of complications or re-intervention); 5) determine the number of pregnancies achieved, depending on the diagnosed anomaly, for a period of 12 months after hysteroscopy performed for diagnostic or surgical purposes.

Methods

The cross-sectional study covered 176 infertile patients with congenital uterine anomalies in which hysteroscopy was performed in the period from January 1, 2013 to anuary 1, 2016. Indications for hysteroscopy in all patients were based on anamnestic data, previous clinical examination, ultrasound examination, and additional methods such as three/dimensional ultrasound and/or hysterosalpingography (HSG). All patients had indication for hysteroscopy. They all had fully laboratory, bacteriological, physical and clinical examinations (including an examination by a anesthesiologist), and then hysteroscopy was performed according to all rules of asepsis.

Hysteroscopy was performed by 5 doctors, which had adequate professional training for endoscopic methods in gynecology and years of experience. It was performed in early proliferative phase of the cycle, with the use of 0.9% NaCl solution as distension media and after the dilatation of cervix to 10.5 mm, hysteroscope with diameter of 10 mm (Karl Storz, Germany) was inserted in the uterus. Mechanical instruments (scissors) and versa-point electrode were used in resection on septum and subseptum, and removing polyps.

In patients with suspected severe congenital anomalies, a laparoscopic intervention was performed along with hysteroscopy in order to establish the exact diagnosis of congenital anomaly, at approximately 30% of the patients. In the case of patients with septum and subseptum in hysteroscopy, a septum was resected. Septum was classified as a longitudinal septum with the length more than 15 mm from the fundus, which divided uterus inside in half. The safety of hysteroscopy, which was assessed on the basis of complications and the need for re-intervention, was observed.

The percentage of pregnancies was observed during the first 12 months after surgery in all patients. Thus, two groups of patients were formed: the first one with patients who became pregnant and the second one with patients who were not pregnant during the observed period. Their characteristics were compared: previous pregnancy, ultrasound and HSG findings, types of congenital anomalies, types of hysteroscopy treatment, and the existence of associated pathology. The following parameters were monitored for all patients: age, professional capacity, type of infertility, duration of infertility, the existence of earlier delivery or abortion, ultrasound findings, findings at hysteroscopy and type of hysteroscopy surgery performed.

The obtained data were processed using the descriptive and analytical statistic methods. Data processing was done using the statistical packages Med Calc 15.8 and SPSS 20.0. From the descriptive statistic method, the arithmetic mean, the standard deviation, the range, the grouping and the tabulation of the data were used. Analytical methods used the *t*-test for numerical data and the χ^2 test for attribute data. The obtained data were analyzed and compared with the results available in domestic and foreign literature. On the basis of the obtained results, the conclusions were brought out in the paper. The study procedure was conducted in accordance with the Helsinki Declaration and approved by the Institutional Ethics Committee.

Results

The study included 176 patients whose basic characteristics are shown in Table 1. The average age of the examined patients was 35 years (from 29 to 47 years). The group of subjects with primary infertility included a total of 107 (61%) of the patients, while 69 (39%) of the patients made thegroup of subjects with secondary infertility. The average length of infertility in the patients tested was 3 years (1–6 years). Only 3% of the patients had previous successful pregnancies. Four patients with one miscarriage had previous *in vitro* fertilization (IVF) process; 8 patients with 2 miscarriages and 2 patients with 3 miscarriage had previously been subjected to IVF process. Fourteen (8%) of all patients had previous one IVF and 2 intrauterine insemination (IUI) processes. In 20% of the subjects the presence of associated pathological changes was observed, such as endometrial polyps, myoms or longitudinal vaginal septum. Polyps were removed at hysteroscopy, myoms were small and also were removed during hysteroscopy, and longitudinal vaginal septum was resected.

Table 1

Characteristics of the study population

Characteristics	Values
Total number, n (%)	176 (100)
Age (years), mean ± SD (range)	34.7 ± 5.5 (21–49)
Infertility, n (%)	
primary	107 (61)
secondary	69 (39)
Duration of infertility (years), mean ± SD	2.8 ± 1.5
Previous childbirth, n (%)	5 (3)
Previous miscarriages, n (%)	66 (38)
1	33 (19)
2	24 (14)
≤ 3	9 (5)
HSG finding, n (%)	52 (30)
normal	7 (4)
uterine anomaly	45 (26)
Associated pathology, n (%)	
polyp	30 (17)
myoma	3 (2)
vaginal septum	2 (1)

HSG – hysterosalpingography.

Complications during hysteroscopy were reported in 2% of the subjects, mainly with complete uterine septum. Repeated hysteroscopy was needed in 5% of the subjects, at resection of a complete septum.

Figure 1 shows distribution of the patients by age. They were grouped in six age groups (in five-year intervals which included the reproductive period of women). Most respondents (34%) were in the age group 30–35 years; in the group of 35–40 years were 30% of the women; the group of 40–45 years included 15% of the patients; the group of 25–30 years had 12%, and the group of 20–25 years had 4% of the patients. Ony 3% of the women were in the age group of more than 45 years.

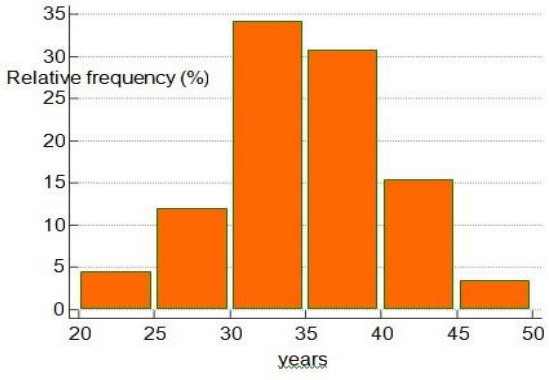

Fig. 1 – Patients age.

Characteristics of respondents who achieved pregnancy after hysteroscopy in observed 12- months period are given in Table 2. Thirty nine subjects had pregnancy; 64% of them were with spontaneous pregnancy, 36% of the patients with

Table 2
Characteristics of patients with pregnancy after hysteroscopy

Characteristics	Patients n (%)
Total number	39 (100)
Spontaneous pregnancy	25 (64)
Medically assisted fertilization	14 (36)
IUI	6 (15)
IVF	8 (21)
Childbirth	33 (85)
spontaneous	28 (72)
cesarean section	5 (13)
Miscarriages	6 (15)

IUI – intrauterine insemination; IVF – *in vitro* fertilisation.

pregnancy underwent the medically assisted fertilization process – 15% of the patients had intrauterine insemination (IUI) and 21% of the patients had IVF. Thirty three respondents successfully terminated pregnancy with children born in a term (28 women had spontaneous delivery and 5 had a cesarean section, while 6 (15%) of the women had miscarriage in the first trimester after hysteroscopy.

Figure 2 shows the type and frequency of uterine anomalies found in hysteroscopy: 76% of the patients had subseptum, 16% had a septum, while rarely presented were *uterus arquatus* (in 4%), *uterus unicornis* (in 3%) and *uterus bicornis* (in 1%).

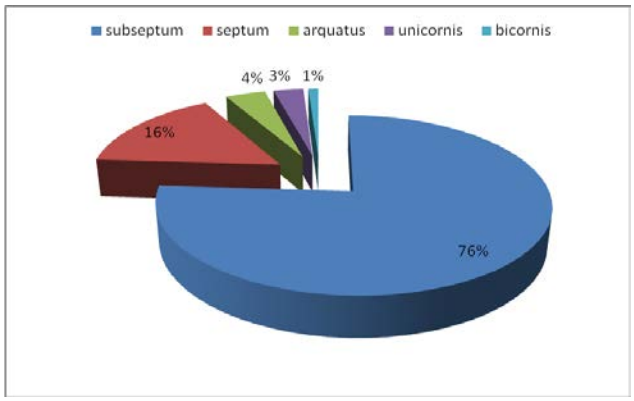

Fig. 2 – Type and frequency of congenital uterine anomalies determined by hysteroscopy.

Figure 3 shows the types of congenital uterine anomalies of the examined women and their frequency in both groups. In the group of subjects who had pregnancy, 28 of them had subseptum, 10 had septum and 1 had *uterus arquatus*. In the group of patients who were not pregnant, the representation of anomalies was: the highest number of respondents (105) had subseptum, 19 had septum, 7 had *uterus arquatus*, 5 had *uterus unicornis* and only one had *uterus bicornis*.

Table 3 shows the data of the patients who conceived after hysteroscopy and those who did not.

The first group consisted of 39 women, only 22% of all respondents involved in study. Their average age was 33

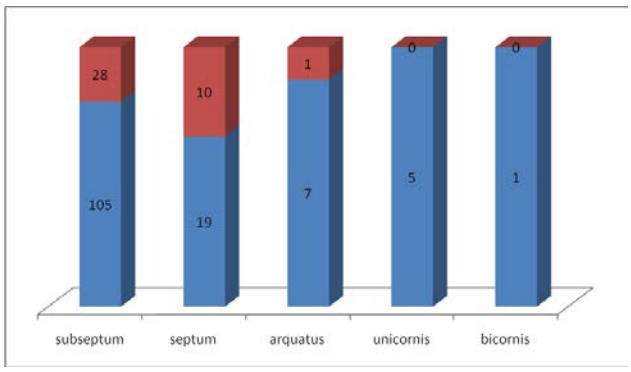

Fig. 3 – The type of congenital anomalies of the uterus in both groups of subjects (red – women with pregnancy after hysteroscopy; blue – women with no pregnancy after hysteroscopy).

years, 44% of them were with primary infertility and 56% with secondary infertility. There were no previous deliveries of women in this group. In this group, 21% of the women had one previous miscarriage, 28% had 2 miscarriages and 8% of the patients had 3 or more miscarriages. The existence of septum and subseptum was diagnosed and resection of changes was made in 97% of the patients. Diagnostic hysteroscopy was performed to verify *uterus unicornis*, associated with diagnostic laparoscopy, in 3% of the women. HSG was performed prior to hysteroscopy in 28% of the women and findings indicated the presence of congenital uterine anomalies.

The second group of respondents consists of 137 women, or about 78% of all respondents. Their average age was 35 years; 66% of the women in this group were with primary infertility and 34% of them with secondary infertility; 4% of the women had previous deliveries; 18% had one previous miscarriage, 10% had 2 miscarriages and 4% had 3 or more miscarriages. The septum and subseptum were diagnosed in 90% of these women and resection of changes was made. Diagnostic hysteroscopy with laparoscopy was performed to verify the *uterus unicornis*, *uterus bicornis* or *uterus arquatus* in 10% of the women in this group. HSG was performed prior to hysteroscopy in 24% of the women and findings indicated the presence of congenital uterine anomalies.

By using the χ^2 test, statistically significant difference ($p < 0.05$) was obtained between the observed groups in the type of infertility, as well as in the number of previous miscarriages. In the group of respondents who had a pregnancy after hysteroscopy, 56% of the subjects were with secondary infertility, and they had a higher percentage of previous miscarriages.

Discussion

The data on the frequency of congenital uterine anomalies as a result of incorrect development of the Miller channels are different, depending on the population included in studies. There are data from the literature, on the incidence of congenital anomalies of the uterus in the general population of about 1%, and even 19% in the

Table 3

Comparison of the groups of patients –with and without pregnancy after operation (OP)

Characteristics of patients	With pregnancy [n = 39 (22.16)]	Without pregnancy [n = 137 (77.84)]	*p*
Age (years), mean ± SD	33.4 ± 4.6	35.0 ± 5.7	0.099
Infertility, n (%)			
primary	17 (44)	99 (66)	0.021
secondary	22 (56)	47 (34)	
Duration of infertility (years), range	1 – 6	1 – 6	0.147
Previous childbirth, n (%)	0 (100)	5 (4)	0.506
Previous miscarriages, n (%)			
1	8 (21)	25 (18)	0.010
2	11 (28)	13 (10)	
< 3	3 (8)	6 (4)	
Hysteroscopy, n (%)			
diagnostic	1 (3)	13 (10)	0.282
resection of septum/sub-septum	38 (97)	124 (90)	
Anomalies, n (%)			
septum /subseptum	38 (97)	124 (91)	0.283
uterus unicornis, uterus arcuatus, uterus duplex	1 (3)	13(9)	
HSG finding, n (%)			
normal	1 (3)	6 (4)	0.661
uterine anomaly	12 (28)	33 (24)	

HSG – hysterosolpingography.

infertile patient population [1]. The true prevalence of uterine anomalies is difficult to estimate because many of them are asymptomatic.

The occurrence of a complete and incomplete longitudinal septum of the uterus occurs in 7–76% of women, depending on the investigated group, which makes it the most common among uterine anomalies. Other anomalies occur in lower percentages (1–10%) and represent anomalies in which hysteroscopy and laparoscopy were performed for the purpose of diagnosis and treatment. The obtained data are similar to those available in the literature. In one of the studies [2], 188 women were examined, with an incidence of anatomical anomalies of 42%, of which *uterus septus* was present in 12%, *uterus bicornis* in 1% and *uterus arquatus* in 0.5% of the women. In the other study [3], the prevalence of the septum in the test population was 7–16%, depending on the classification, after ultrasound examination. In the third study [4] with infertile patients, incidence of the subseptum and septum was 60% and 40%, respectvely. In the fourth similar study [5], 287 women had diagnosed anomalies of the uterus: *uterus septus* (in 55%), *uterus arquatus* (in 14%), *uterus bicornis* (in 10%), *uterus unicornis* (in 6%), and only 2% of the patients had *uterus septum* with double cervix.

In our study, the mean age of the examined patients was 35 years, ranging from 20 to 49 years. Similar results were published by other authors [6, 7].

The average duration of infertility in our patients was3 years (range 1–6 years). In available data from other studies, the duration of infertility was more than 2 years [6].

Only 3% of our respondents had previously successful pregnancies, while 38% of the respondents had previously miscarriages (19% had 1 miscarriage, 14% 2 miscarriages and 5% three or more miscarriages). Similar data are available in the literature, and the percentage of births in the examined group before the hysteroscopic operation was 5% [8]. During the first trimester, the risk of spontaneous abortion in patients with uterine septum is 28–45%, according to literature data [2, 9, 10], and the cause of weaker vascularization of the implant site is considered to be the cause.

Today, we use routine resection of the septum during hysteroscopy. Retrospective cohort studies performed on women who underwent hysteroscopic resection of the uterine septum indicate a significant reduction in the abortion rate from 67% to 13% [7, 9] and an increase in the live-birth rate to 37% [7, 11]. According to literature data, the percentage of pregnancy after hysteroscopic resection is 43% [12] and even 69% [13] with an incidence of live births of 49% [13]. In a similar study, patients with an idiopathic infertility were diagnosed with uterine septum; 12 months after the hysteroscopic resection, 44% of the subjects achieved pregnancy, and in 37% pregnancy ended in live births [11]. Some authors questioned the justification of hysteroscopy after 2 miscarriages [10].

The scientific community carried out a reassessment of the clinical significance of hysteroscopy in the diagnosis and treatment of uterine factors and the role in the treatment of infertility, thanks to its potential for improving reproductive results and reducing time to pregnancy [14]. Studies can also be found to investigate the possibility of diagnosing and treating uterine septum only on the basis of hysteroscopy, which implies that only the use of hysteroscopy is insufficient [7, 15], without prior detailed preparation, previous clinical examination, ultrasound measurement and review of additional methods such as three dimensional (3D) ultrasound technique (3D), HSG or magnetic resonance (MR) [15].

According to the available data, the success of 3D ultrasound in diagnosis of uterine anomalies ranges from 96%–100% [16, 17], which makes this technique recommended for routine use as accurate and precise [18–20]. Additional diagnostic method, such as HSG, are less commonly used for diagnosis of congenital uterine anomalies. In our study, HSG was applied in 30% of the patients, and in 24% of the patients, HSG-diagnosed anomalies were confirmed by hysteroscopy, while 6% of the patients had false-negative results. Some authors have concluded that HSG is not an optimal method for diagnosing uterine anomalies (because of the impossibility of visualizing the outer contour of the uterus), while the 3D ultrasound technique may be an alternative method for MR [21]. According to literature data, MR is successful in 29–60% of the cases in the diagnosis of congenital uterine anomalies [16, 20].

Complications of hysteroscopy were recorded in 2% of our subjects, mainly those with complete uterine septum. Repeated hysteroscopy were needed in 5% of the patients, in the resection of the complete septum. According to literature data, the percentage of re-intervention is 2% [4, 22]. There are also available data on 64 patients with hysteroscopic septum resection of which 3% had complications such as the uterine wall perforation [13]. In other studies, the overall percentage of intraoperative/postoperative complications and re-intervetions was 1.7% and 6%, respectively 6% [13].

In 20% of the subjects in our study, the presence of associated pathological changes was observed, of which polyps were present in 17% of the subjects, myomas in 2%, and septum of the vagina in 1% of the subjects. In available literature data, 8% of infertile patients with recurrent abortion, were diagnosed with myoma by hysteroscopy [23].

In our study, 39 (22%) of the patients experienced pregnancy after hysteroscopy in the observed period of 12 months. In a study similar to ours, out of 88 respondents in the observed period of 12 months after hysteroscopy resection, 41% of the respondents had pregnancy [6]. In other studies, the observed period was on average 12 up to 68 months, with the overall percentage of pregnancies being 60%, and the percentage of live births being 45% [13].

In our study, pregnancy occurred spontaneously in 64% of all pregnancies and 36% occurred after the medically assisted fertilization process. Besides, 33 (85%) of the women had pregnancy with child born in a term, out of which 28 women had spontaneous delivery and 5 women had surgical deliveries. Six (15%) of the pregnancies ended with miscarriage. According to the literature data, 61% of the patients have been pregnant after hysteroscopy resection, with 25 pregnancies (13 from the IVF process, i.e. more than half) [24]. Also, the data of other authors indicate that percentages of successful pregnancies after hysteroscopy are increased by IVF processes [25]. Therefore, some authors advise the routine use of office hysteroscopy as the basic method preceding the IVF/ intracytoplasmic sperm injection (ICSI) attempt, even in women with correct results in transvaginal ultrasound examination [26].

In the literature, 63% of the respondents experienced pregnancy after hysteroscopic septum resection, while 56% of the subjects reported pregnancy in *uterus arquatus* [8]. Data from other studies indicate an improvement in fertility following hysteroscopic resections in other uterine anomalies, except in *uterus bicornis* [27]. Data in some studies indicate that there is no statistically significant difference in pregnancy outcomes after hysteroscopic resection in *uterus septum* and *uterus arquatus* [28]. Also, the incidence of complete and incomplete uterine septum among patients is dominated by the available data [24].

Using the χ^2 test, statistically significant difference ($p < 0.05$) was obtained between the observed groups in the representation of the type of infertility, as well as in the number of previous abortions. In the group of the women who had a pregnancy after hysteroscopy, 56% of them were with secondary infertility, and they had a higher percentage of previous miscarriages. Similar data on the association of previous miscarriages with successful pregnancy after hysteroscopic septum resection can be found in other clinical studies [29, 30]. Data on various techniques of resection and outcome of surgery for later pregnancies and delivery of children at birth are available [31–33]. It was shown that there was an increase in the percentage of pregnancies by using IVF/ICSI processes in women with uterine anomalies either treated or not treated by hysteroscopy [32]. Women with congenital uterine abnormalities have poorer reproductive outcomes (a greater percentage of miscarriages in the first trimester, higher preterm birth rates, less pregnancy), regardless of whether they have spontaneous pregnancy or pregnancy induced by medically assisted fertilization, compared to women with normal uterus [33]. Some studies also dealt with the problem of additional therapy after hysteroscopy [34]. According to available literature data, the worst prognosis for pregnancy have women with *uterus unicornis*, while those with uterine septum (complete or incomplete) have the highest miscarriage rate in the first trimester [33, 35].

Limitation of study

This study has some limitations. The study included women who were diagnosed with infertility according to valid criteria. The study had a relatively small number of subjects in one of the reference centers for hysteroscopic treatment of uterine anomalies. Since hysteroscopy was conducted only on the basis of indications, some women were admitted after one or more miscarriages and one or more unsuccessful processes of IVF. Some of the patients after hysteroscopic treatment and 12- months period of the control at the Clinic, had back to their hometowns, and did not get back to the Clinic in the next pregnancy and we did not know exactly how many of them had pregnancy and its outcomes after the 12-month period. Data of husbands/partners' infertility were not followed at all patients, so they were excluded from the study. It was recommended that center made some follow-up program

for the patients which had been diagnosed with congenital uterine anomalies and had laparoscopic, hysteroscopic or non-endoscopic operative treatment, so that doctors could follow them during pregnancy and its outcome.

Conclusion

The most common congenital uterine anomalies in our study were incomplete and complete uterine septum (subseptum in 76% and septum in 16% of the patients). Other congenital uterine anomalies were rare: *uterus arquatus* in 4%, *uterus unicornis* in 3% and *uterus bicornis* in 1% of the patients. A small number of patients with congenital anomalies (only 3%) had previously successful pregnancies completed by childbirth in a term. More than one-third (38%) of the respondents had data of previous miscarriage in the first trimester.

Hysteroscopy was shown to be a safe endoscopic method allowing the resection of longitudinal septum and subseptum. The complications were present in 2% of the patients with hysteroscopy performed, and re-interventions were made in 5% of the subjects (in the cases of a complete septum of the uterus).

More than 20% of the patients had a pregnancy after hysteroscopy in the observed period of 12 months, while in most of them, pregnancy was completed by childbirth in a term (85%). Pregnant women were mostly diagnosed with septum and subseptum of the uterus and hysteroscopy resection of changes was made. The percentage of miscarriages in the women decreased from 38%, before hysteroscopy to 15%, after hysteroscopy.

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The effect of moral foundations and personality dimensions of health workers on patient satisfaction with healthcare services

Uticaj moralnih osnova i dimenzija ličnosti zdravstvenih radnika na zadovoljstvo bolesnika zdravstvenom uslugom

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Abstract

Background/Aim. Working with people in medical practice requires knowledge of the basic principles of personality psychology. The aim of this research was to examine the influence of moral foundations and personality dimensions of health workers on patient satisfaction with health service, as well as determining the factors influencing their satisfaction. **Methods.** The research was conducted using the Big Five Inventory (BFI), theMoral Foundations Questionnaire (MFQ) and the Questionnaire of patient satisfaction with healthcare service, from October 2014 to March 2016. The survey involved 693 respondents: 329 healthcare workers (44 males and 285 females) and 364 patients (154 males and 210 females). **Results.** With the increase of the openness of healthcare professionals, the patients' satisfaction was also growing. Regarding moral foundations of healthcare workers, there was an opposite trend – with lower authoritativeness of healthcare workers, patient satisfaction was greater. Finally, with more pronounced purity of healthcare workers there was a decrease in the level of patients' satisfaction with the received healthcare service. **Conclusion**. There is a direct association between the moral foundations and personality dimensions of health workers, and patients' satisfaction with healthcare service. Thus, any strategy for improving the quality of healthcare service should take into account personal characteristics of healthcare workers. Healthcare professionals are expected to fulfill their life and professional tasks in as a human way as possible, since preservation of the health and helping to overcome an illness, in case it develops, are the basic values of every human being. It should result in more clearly defined priorities for improvement of the quality of work of healthcare workers.

Key words:
health services; health personnel; morals; patient satisfaction; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Rad sa ljudima u medicinskoj praksi zahteva poznavanje osnovnih principa psihologije ličnosti. Cilj ovog istraživanja je bio sagledavanje uticaja moralnih osnova i dimenzija ličnosti zdravstvenih radnika na zadovoljstvo bolesnika zdravstvenom uslugom, kao i utvrđivanje faktora koji utiču na to. **Metode.** Istraživanje je sprovedeno primenom upitnika *Big Five Inventory* (BFI), *Moral Foundations Questionnarie* (MFQ) kao i Upitnik zadovoljstva bolesnika zdravstvenom uslugom u periodu od oktobra 2014. do marta 2016. godine. U istraživanju je učestvovalo 693 ispitanika: 329 zdravstvenih radnika (44 muškog i 285 ženskog pola) i 364 bolesnika (154 muškog i 210 ženskog pola). **Rezultati.** Sa porastom otvorenosti zdravstvenih radnika raste i zadovoljstvo bolesnika. Posmatrajući moralne osnove zdravstvenih radnika, postoji trend da što je niža autoritativnost zdravstvenih radnika, zadovoljstvo bolesnika je veće. Konačno, izraženija čistota morala kod zdravstvenih radnika, dovodi kod bolesnika do opadanja stepena zadovoljstva dobijenom zdravstvenom uslugom. **Zaključak.** Postoji direktna veza između moralnih osnova i dimenzija ličnosti zdravstvenih radnika i zadovoljstva bolesnika zdravstvenom uslugom. Stoga se, u okviru bilo koje strategije za unapređenje kvaliteta usluga u zdravstvenim sistemima, mora voditi računa o personalnim karakteristikama zdravstvenih radnika. Od zdravstvenih radnika se očekuje da na što humaniji način ispune svoj životni i profesionalni zadatak, jer je očuvanje zdravlja, kao i pomaganje u prevazilaženju bolesti, ukoliko do nje dođe, osnovna vrednost svakog čoveka. Time se mogu jasnije definisati prioriteti za poboljšanje i unapređenje kvaliteta rada zdravstvenih radnika

Ključne reči:
zdravstvene ustanove; zdravstveno osoblje; moral; bolesnik, zadovoljstvo; ankete i upitnici.

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Introduction

Assessment of users' satisfaction is one of the basic elements of improving the quality of work of healthcare institutions and a prerequisite for a quality of health care [1]. In order to motivate health professionals for the targeted activities it is necessary to know the basic principles of motivation psychology and mechanisms for developing internal motivation, which should be acted on all employers as well as on the level of the State. Patients' satisfaction could be used as an instrument for measuring the success of a healthcare institution and for improving the quality of health care itself [2]. The programs of satisfaction of the users of healthcare services are studied and applied in the health sector as instruments for achieving patients' satisfaction [2]. The aim of this research was to examine the influence of moral foundations and personality dimensions of healthcare professionals on patients 'satisfaction with healthcare service in primary healthcare institutions.

In accordance with the above stated and taking into account the existing literature, the influence of following personality dimensions (tested by the Big Five Inventory – BFI) and moral foundations (tested by the Moral Foundation Questionnaire – MFQ) on patients' satisfaction with healthcare service were determined: the extraversion dimension (BFI1), the neuroticism dimension (BFI2), the openness dimension (BFI3), the agreeableness dimension (BFI4), the conscientiousness dimension (BFI5), and care (MFQ1), fairness (MFQ2), loyalty (MFQ3), authoritativeness (MFQ4) and purity (MFQ5), respectively.

Results of the research should form the basis for planning activities that would improve the quality of work of healthcare professionals and patients' satisfaction as the main output.

Methods

The research was conducted as a survey, by distributing questionnaires with a prior consent of the Ethics Committees of the health centers "Novi Sad", "Kula" and "Dr. Đorđe Lazić". The Health Center "Novi Sad", the largest health center in the region, with the largest number of employees (1,465), with 33 facilities (some of which are in urban and some in suburban settlements), and the largest number of services provided (8,835,567 in 2016) and the most intensive patient turnout (of all ages – from the youngest, to the working age and pensioners), was taken as a representative one. In Novi Sad, there is no general hospital as a secondary-level healthcare institution, expect the Military Hospital in Petrovaradin; for that reason the Health Center "Novi Sad" plays a major role in bridging the gap between the primary and tertiary level of health care.

Research instruments

Three instruments were selected in accordance with the research objectives and based on the theoretical and empirical backgrounds [13]. The first one was the Big Five Inventory, one of personality testing questionnaires, developed by Italian authors Caprara et al. [4]. It consists of 44 statements, to which a respondent answers on the Likert scale ranging from 1 to 5 (Cronbach alpha = 0.857 in this research). The second one was the Moral Foundations Questionnaire, which consists of 32 statements, divided into two groups (Cronbach alpha = 0.893 in this research), and the answers were given in the Likert scale format in the range of 1 (not very relevant) to 5 (strongly relevant), and ranging from 1 (disagree strongly) to 5 (agree strongly). The third instrument was the Patient satisfaction questionnaire, and an *ad hoc* questionnaire constructed only for the purposes of this research, which consisted of 25 questions, (Cronbach alpha = 0.752 in this research) with answers offered in the Likert scale format, ranging from 1 (not at all relevant) to 5 (extremely relevant). According to the Cronbach alpha coefficients we could say that instruments we used in this research were reliable.

One independent study panel consisted of health workers from the three medical institutions, while the other panel included patients treated in these institutions [5]. Firstly, it was necessary to translate and culturally adapt the selected instruments. Two translators, whose mother tongue was Serbian and who were familiar with the research, independently translated both questionnaires from English into Serbian. The two versions were compared, and after back-translation and final correction and validation by a university professor in psychology and management, the versions were finally accepted. The questionnaires were anonymously filled in order to obtain as sincere responses as possible.

Study sample

The survey was conducted in the period from October 2014 to March 2016. A total of 1,000 questionnaires were distributed, 758 were returned, of which 693 were valid. Of these, 329 respondents were health workers (154 doctors and 175 nurses, 44 were male and 285 female), and 364 were patients (154 were male and 210 female). In order to confirm or reject the research hypotheses, the obtained results were subjected to the structural equation modeling (SEM) analysis. The analysis was performed in the WARPLS 4.0 program because it allows determining also non-linear relationships between variables included in the structural model.

Results

Data obtained from patients are shown in Table 1. Out of the total number of 364 patients treated in the studied healthcare centers, the largest number stated that they were the most satisfied when a doctor was well organized (4.64), then spontaneous (4.50), attentive and open (4.41). While patients reportedly disagreed with the statement that medical staff had violated their national or social rights (2.10), they were not satisfied with the lenght of time they spent on examinations (2.32), and they did not agree with the statement that healthcare services were equally accessible to all (2.76).

Table 1

Factors of patients' satisfaction with healthcare service

Factors of satisfaction	Average score
I appreciate when the doctor is well organized	4.64
I appreciate when the doctor is spontaneous	4.50
I appreciate when the doctor is attentive and open	4.41
I would characterize myself as a communicative patient	4.31
I appreciate more when the doctor is committed than distanced	4.16
The doctor listens to me carefully during the examination	3.95
With a nurse I feel there is willingness to help	3.93
I would characterize myself as a responsible patient	3.90
There is a lot of corruption in the medical profession	3.84
I am satisfied with the maintenance of personal data	3.68
Your doctor is making effort to gain your trust	3.63
With a doctor I feel empathy and understanding because of the illness	3.48
You get immediate service with emergency health problems	3.36
You wait for a long time in the waiting room regardless of the appointment	3.33
I am satisfied with the costs of some medical examinations	3.04
I am satisfied with home treatment services	3.01
I am satisfied with the availability of the Protector of patients' rights	2.89
I am satisfied with the organization of preventive examinations in some departments	2.88
Health is equally accessible to all	2.76
How long does averagely the examination take at the doctor's office	2.32
Medical staff has once violated your national or social rights	2.10

Table 2

Health workers' personality dimensions in relation to professional position

Healthcare workers	Personality dimensions of healthcare workers				
	Extroversion (BFI1)	Neuroticism (BFI2)	Openness (BFI3)	Agreeableness (BFI4)	Conscientiousness (BFI5)
Doctors (n = 154)	5.02	3.96	5.05	4.64	5.66
Nurses (n = 175)	5.11	3.96	5.07	4.66	5.66
Total (n = 329)	5.07	3.96	5.06	4.66	5.66

BFI – Big Five Inventory

As shown in Table 2, both doctors and nurses score was highest on the dimension of conscientiousness (5.66) and lowest one on the dimension of neuroticism (3.96).

As shown in Table 3, healthcare workers have the most prominent dimension of empathy for patients (29.32 for doctors and 29.29 for nurses), and the least significant

Table 3

Moral foundations of healthcare workers in relation to their position

Healthcare workers	Personality dimensions of healthcare workers				
	Care (MFQ1)	Fairness (MFQ2)	Loyalty (MFQ3)	Authority (MFQ4)	Purity (MFQ5)
Doctors (n = 154)	29.32	28.77	28.16	28.13	28.58
Nurses (n = 175)	29.29	28.89	28.25	28.13	28.58
Total (n = 329)	29.31	28.84	28.21	28.13	28.58

MFQ – Moral Foundation Questionnaire

Table 4

Basic parameters of structural equation modeling (SEM) analyses

Model fit and quality indices	*p*
Average path coefficient (APC) = 0.083	0.027
Average R-squared (ARS) = 0.062	0.058
Average adjusted R-squared (AARS) = 0.035	0.124
Average block VIF (AVIF) = 1.167, acceptable if < = 5, ideally < = 3.3	
Average full collinearity VIF (AFVIF) = 1.727, acceptable if < = 5, ideally < = 3.3	
Tenenhaus GoF (GoF) = 0.243, small > = 0.1, medium > = 0.25, large > = 0.36	
Sympson's paradox ratio (SPR) = 0.700, acceptable if > = 0.7, ideally = 1	
R-squared contribution ratio (RSCR) = 0.871, acceptable if > = 0.9, ideally = 1	
Statistical suppression ratio (SSR) = 0.900, acceptable if > = 0.7	
Nonlinear bivariate causality direction ratio (NLBCDR) = 0.800, acceptable if > = 0.7	

Source: WARPLS 4.0 program.

Table 5

Results of structural equation modeling (SEM) analysis

Parameters	BFI1	BFI2	BFI3	BFI4	BFI5	MFQ1	MFQ2	MFQ3	MFQ4	MFQ5
Path coefficient	0.01	0.058	-0.145	0.068	-0.064	-0.024	-0.070	0.007	0.154	0.137
p	0.021	0.132	0.003	0.095	0.110	0.321	0.090	0.445	0.001	0.004

BFI – Big Five Inventory (for explanation see Table 2);
MFQ – Moral Foundation Questionnaire (for explanation see Table 3).

dimension of authoritativeness (28.13 for doctors and 28.13 for nurses).

The basic parameters of the SEM analysis presented in Table 4 show that the suggested model was statistically significant and that all relevant parameters suggesting significance of the analysis were within the limits that make the model acceptable.

The obtained results indicated that openness ($p = 0.14$, $p < 0.01$), as well as authoritativeness ($p = 0.15$, $p < 0.01$) and purity ($p = 0.14$, $p < 0.01$) of health workers significantly affected patients' satisfaction with the quality of healthcare services (Table 5). Other personality dimensions and moral foundations of health workers did not show statistical significance.

Discussion

The healthcare service quality can be defined as the degree to which the healthcare service for an individual and a population increases the likelihood of the desired health outcomes, although it is consistent with the current professional knowledge [6]. The categories of healthcare quality indicators such as equipment, facilities, human resources and qualifications were originally developed by Donabedian [7], while Lohr and Schroeder [6] spent many years devising and expanding this scheme. It is important to keep in mind that researchers suggested that these variables are not a direct measure of quality. Instead, resources are there only to help us determine whether the quality is good or not.

The originality of this research lays in establishing direct connections between moral foundations and personality dimensions of healthcare workers and patient satisfaction with primary healthcare service. By analyzing the data, we noticed that if the healthcare worker is more open, the patient is more satisfied. The characteristic of openness is defined by attention to inner feelings, preferences of diversity, enthusiasm, originality, broad interests, and if these characteristics are more pronounced among health workers, patients are more satisfied. Analyzing the authoritativeness factor, which was distinguished by the SEM analysis, it could be noticed that with lower authoritativeness of healthcare workers, patients' satisfaction increases. This moral foundation is formed on the basis of the long history of primates and hierarchical social interactions, based on the virtues of leadership, including respect for the rule of laws and respect for tradition [8]. Healthcare workers who tend to violate strict rules and norms, and oblige patients have more satisfied patients, as opposed to those who act strictly according to the rules and do not oblige patients. Frequently, strictness of healthcare workers leads themselves to an undesirable situation, when while respecting the rules they cannot meet every patient's expectations. Analyzing the factor of purity of healthcare workers, it was found that the trait of purity was in a negative correlation with patients' satisfaction. This moral foundation is based on the psychology of disgust and contamination. These are basically religious concepts which lead us to aim to live in a sublime, less physical and kinder world. It advocates the idea that the body is a temple that can be desecrated by immoral activities [8]. Purity primarily refers to the respect of the Hippocratic Oath, which implies that health workers should help everyone who asks for help, regardless of religious, gender and national affiliations. Healthcare workers who work according to established standards and norms, without undue concessions, cause less patients' satisfaction. Healthcare workers who work outside established standards, who want to grant sick leave although they consider it unnecessary and who are prone to corruption, cause more satisfaction with patients. Although

this is in disagreement with literature data, the practice has shown a positive correlation between these phenomena. All this is due to the fact that many patients do not visit a doctor because they are really sick, but for the reasons of getting a sick leave, obtaining documents for going to a disability pension, or getting medical records for the need of collecting medical insurance. Moral psychology is a rapidly growing field, yet progress is limited by the quality and availability of existing measures. The effects of emotion and cognition on moral judgment or investigating the neurological basis of moral judgment demands a validated, standardized stimuli set that covers the moral domain [9].

Similar results were published by Huseinspahić [10], who investigated the effect of quality of health care as a prerequisite for patients' satisfaction. He also partially confirmed that the level of patients' (dis)satisfaction is a result of the overall perception of the quality of healthcare services, i.e. confirmation or non-assertion of preferences in terms of the technical and functional dimensions of the quality of healthcare services [10]. Also, results published by Shan et al. [11] show that patients' satisfaction with health care is low in Heilongjiang in China. The fundamental problem of the poor satisfaction of patients is the lack of trust. In addition, inequality of a protector of patients' rights, and perceived poor quality of services also contribute to patients' dissatisfaction with health care. Therefore, reforming the current medical insurance system, as well as enhancing healthcare service quality will be key to address the problems of distrust [11, 12]. From the results of this research, as well as from the research in the region and on the other continents, it is clearly seen that there is a problem in the healthcare system, which should be improved by the state in order to make it better, and patients more satisfied. The medical standard generates conflict zones and ethical dilemmas, while the freedom of choice over the type of medical procedure preferred makes a reference to the manner of understanding, acceptance and application of the new [13]. Ethics comes with the message of the concept of health from a personal issue, limited to patients' satisfaction or dissatisfaction [14].

Limitations and directions for further research

The research should be carried out on a larger sample with respondents from the whole the Republic of Serbia and by using other instruments, as well as it should be extended to the secondary and tertiary levels of health care.

On the basis of the mentioned limitations, proposals for further research are suggested: to examine the motivation and emotional status of healthcare workers; to examine how the emotional characteristics of the service providers affect the quality of services in the health systems and to use other questionnaires for this purpose; to conduct a research in which receivers of healthcare services would assess the expertise of health providers; to conduct a survey in which healthcare workers would report their satisfaction with their work and correlate the findings with patients' satisfaction; to examine the ethics of healthcare workers in cases of patients' taking sick leave.

Conclusion

There is a direct association between the moral foundations (authority and purity) and personality dimensions (openness) of healthcare workers, and patients' satisfaction with healthcare service. Thus, any strategy for improving the quality of healthcare services should take into account moral and personal characteristics of healthcare workers. The research put emphasis on interpersonal relationships, since humanity is one of the main prerequisites of a good healthcare system and quality of health care.

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Assessment of supracrestal tissue attachment variation in patients with chronic periodontitis before and after treatment: A clinical-radiographic study

Procena varijacija pripoja suprakrestalnog tkiva kod bolesnika sa hroničnim periodontitisom pre i posle lečenja: kliničko-radiografska studija

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Abstract

Background/Aim. Healthy periodontium comprises the dento-gingival junction. Periodontal disease starts to appear when the integrity of the junctional epithelium is disturbed. Assessment of the supracrestal tissue attachment (SCTA) is essential because there is a frequent need for restoration or prosthesis after periodontal surgical and non-surgical therapy. The aim of the present study was to evaluate the SCTA variations in a patients with chronic periodontitis before and after treatment. **Methods.** Thirty systemically healthy patients with periodontitis were enrolled in the study. Fifteen patients were subjected to scaling and root planing and 15 to open flap debridement. Radiographic and clinical findings of the SCTA were assessed before and after treatment at 3-month and 6-month intervals. **Results.** Comparison between clinical and radiographic findings of the SCTA showed a significant difference in patients with periodontitis ($p < 0.05$). This difference was not significant after treatment of patients with shallow pockets with scaling and root planing ($p > 0.05$), but showed a significant difference in patients with moderate pockets treated by open flap debridement ($p < 0.05$). **Conclusion.** Progression in periodontal disease causes a reduction in the SCTA dimension, which regains its original dimensions after periodontal therapy. It takes around 3 months for the shallow pockets to regain the supracrestal tissue attachment to the original dimension when treated by scaling and root planing, whereas moderate pockets regain it after 6 months when treated with open flap debridement.

Key words:
periodontics; debridement; surgical flaps; gingival recession; dental scaling; radiography, dental.

Apstrakt

Uvod/Cilj. Zdravi periodoncijum podrazumeva zubno-gingivni spoj. Periodontalna bolest počinje da se javlja kada se poremeti integritet spoja epitela. Procena suprakrestalnog tkivnog spoja (SKTS) je od velikog značaja jer često postoji potreba za restorativnim ili protetičkim radovima nakon hirurgije periodoncijuma ili nehirurške terapije. Cilj ove studije bio je da se procene varijacije SKTS kod bolesnika sa hroničnim periodontitisom pre i posle lečenja. **Metode.** Trideset zdravih bolesnika sa periodontitisom bilo je uključeno u studiju; 15 bolesnika je bilo podvrgnuto klasičnom, nehirurškom lečenju periodontitisa, a 15 režanj operaciji. Suprakrestalni tkivni spoj procenjivan je radiografski i klinički pre i tri i šest meseci posle lečenja. **Rezultati.** Poređenje između kliničkog i radiološkog nalaza SKTS pokazalo je značajnu razliku kod bolesnika sa periodontitisom ($p < 0,05$). Ova razlika nije bila značajna nakon lečenja bolesnika sa plitkim džepovima tretiranih na klasičan, nehirurški način ($p > 0,05$), ali je bila značajna razlika kod bolesnika sa umerenom dubinom džepova lečenih režanj operacijom ($p < 0,05$). **Zaključak.** Progresija periodontalne bolesti dovodi do smanjenja dimenzija SKTS koji se normalizuje nakon lečenja periodoncijuma. Kod postojanja plitikih džepova, potrebno je oko tri meseca da

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se postigne prvobitna dimenzija SKTS primenom klasičnog, nehirurškog metoda lečenja. Kod umereno dubokih džepova, normalna dubina STS postiže se šest meseci posle režanj operacije.

Ključne reči:
periodontologija; debridman; režnjevi, hirurški; gingiva, povlačenje; zub, uklanjanje mekih i tvrdih naslaga; radiografija, stomatološka.

Introduction

Periodontium is a subject to morphologic and functional variations, as well as changes associated with age [1]. Therefore, changes taking place in one of the periodontal component may have significant consequences on other components with respect to maintenance and regeneration [2]. The junctional epithelium forms a collar around the cervical portion of the tooth. The role of junctional epithelium is especially crucial because it seals off periodontal compartment from the oral environment [3]. The attachment of the junctional epithelium to the tooth is reinforced by gingival fibers, referred to as the dento-gingival unit [4]. Attachment loss happens when junctional epithelium starts to migrate apically – this is a best example of how structure regulates function [3].

Dento-gingival junction to the tooth surface is composed of a fibrous, supracrestal connective tissue attachment and an epithelial attachment (junctional epithelium) [5]. This anatomical structure has been termed as "Supracrestal tissue attachment" [6] (SCTA) previously known as "biological width" and introduced as an important concept in periodontics and restorative dentistry [7]. Histologic dimensions of the SCTA were comprehensively evaluated on teeth from autopsy specimens of subjects 19 to 50 years of age, having an average width of 1.07 mm for connective tissue and 0.97 mm for the junctional epithelium. These dimensions varied considerably with age and level of apical migration of the epithelial attachment [5].

One of the first changes in periodontitis is migration of the junctional epithelium along the root surface, resulting in formation of a periodontal pocket. The ensuing inflammatory response leads to the degradation of the underlying connective tissue, first around blood vessels and then spreading into adjacent regions, resulting in structural and functional disintegration of the gingiva [3].

The SCTA is an important, but variable component of the periodontal support, which may provide periodontal stability to teeth that lack alveolar bone support, as well as providing an unusually large SCTA [8]. Considerable variability has been shown to exist in the SCTA dimensions in cross-sectional studies of autopsy materials with no overt periodontal pathology [5, 9]. Various clinical and experimental studies are available in the literature, investigating the effect of the periodontal surgical procedure on healing and regeneration of the SCTA[10–12]. Very few studies, however, have been done on measurement of the SCTA before and after flap surgery in humans with periodontal pockets [13, 14]. So, the purpose of this study was to determine the SCTA variation in patients with chronic periodontitis and pockets before and after treatment.

Methods

The study design was reviewed and approved by the Ethical Review Board of the institution. The patients (mean age 38 ± 10.57 years) meeting the selection criteria were randomly recruited from the out-patient Department of Periodontology and Implantology. The study design was explained to the patients and informed consents were obtained. Patients with at least 20 teeth in the oral cavity, the presence of periodontal pockets in the range of 3–7 mm in at least 10 teeth and with radiographic evidence of horizontal bone loss were included in the study. Patients diagnosed with aggressive periodontitis, angular osseous defects, tobacco users, pregnant and lactating women, systemically compromised, and for whom surgery is contraindicated were excluded from the study.

Thirty selected patients were divided into two groups, depending on the selected criteria. Study group 1 consisted of patients with periodontal pockets in the range of > 3 to < 5 mm. This group underwent non-surgical periodontal therapy comprising of scaling and root planing (SRP), performed under local anesthesia. Study group 2 consisted of patients with periodontal pockets in the range of ≥ 5 to 7 mm. This group underwent surgical periodontal therapy comprising of scaling and root planing (SRP) followed by periodontal flap surgery (PFS) under local anesthesia (Figure 1).

Dental radiographs of the study teeth were made and their pre-operative clinical presentation was photographically documented. Occlusal stents for positioning the wire pins were fabricated with self-cured acrylic resin. They were used to measure the different clinical parameters and recorded to the nearest millimeter (Figure 2).

Baseline measurements were done clinically and radiographically. Clinical examination was done in the buccal and lingual gingival sulcus. Radiographic examination was done in the mesial and distal gingival sulcus. The clinical parameters were recorded as: Probing Pocket Depth (PPD) – measured as the distance from the gingival margin to the fundus of the periodontal pocket by using the University of Michigan "O" probe William marking (Hu-Friedy Mfg. Co, Chicago, Illinois, United States); Probing Bone Level (PBL) – measured as the distance between the gingival margins to the crest of the bone by bone sounding under local anesthesia; Clinical Supracrestal Tissue

Attachment (C-SCTA) – calculated as the difference of the above two measurements. Parameters were recorded at

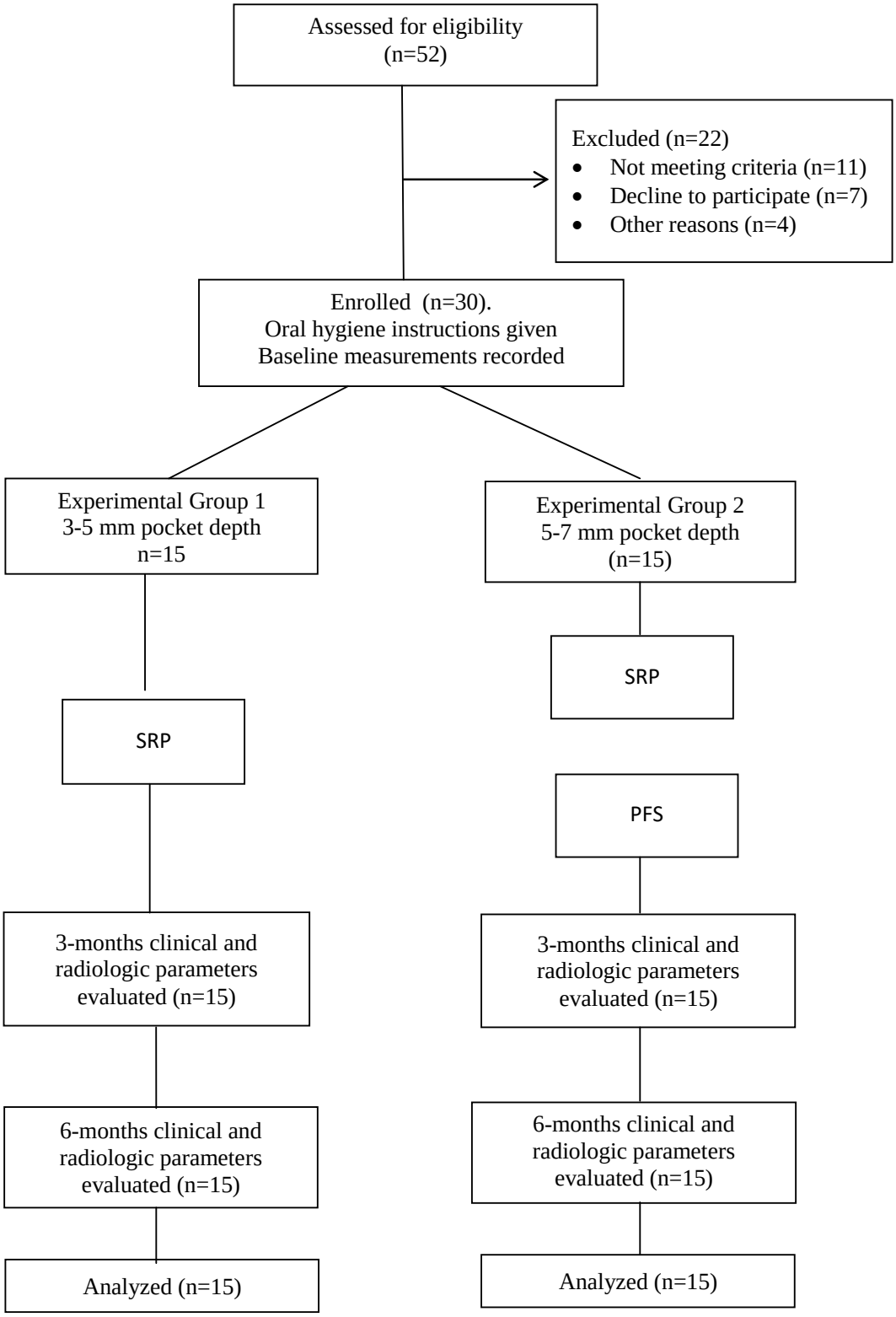

Fig. 1 – Flow chart of the study design.
For abbreviations see under Table 1.

baseline, 3 months and 6 months after the selected treatment for the assessment of the SCTA.

Standardized radiovisiographs (RVG) (Drsuni™ Digital Imaging software, San Jose, CA, USA) were taken with the RINN XCP system™ (Dentsply Sirona, Pennsylvania, USA) at baseline, and 3 months and 6 months postoperatively. Radiographs were standardized by using bisecting angle technique with a film holder device. Radiographs were taken after inserting a radiographic marker (wire pin) into the mesial and distal gingival sulcus while keeping the acrylic

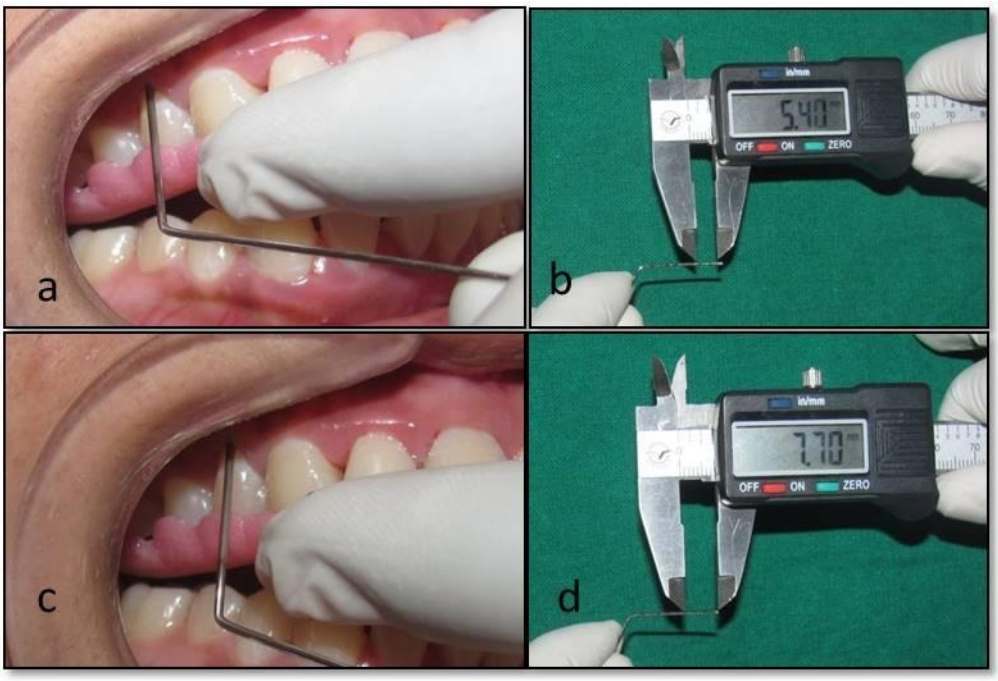

Fig. 2 – Calculation of clinical supracrestal tissue attachment: a) Wire pin placed in pocket; b) Pocket depth measured using vernier caliper; c) Bone sounding; d) Bone level measured using vernier caliper.

Fig. 3 – a) Radiograph with wire pins placed interdentally; b) Interdental supracrestal tissue attachment measured.

stent in position (Figure 3). The distance was calculated and measured by the radio-visiographic analyzing tool. Landmarks were identified on the radiographs as: Radiographic Supracrestal Tissue Attachment (R-SCTA) – it was measured as the distance from the apical tip of the radio-opaque marker to the alveolar crest; Alveolar Crest (AC) – it was defined as the crossing of the silhouette of the interdental bone with the root surface. The average of the mesial and distal scores was the R-SCTA. A total Supra Crestal Tissue Attachment (T-SCTA) was calculated by making the average sum of C-SCTA and R-SCTA.

The patients were divided into two groups: the patients in the study group 1 underwent non-surgical periodontal therapy comprising of scaling and root planing. Patients were excluded from the study group 2, if the residual pockets were less than 5mm after phase I therapy. After re-evaluation, patients underwent conventional periodontal flap surgery (Split papilla flap), which was performed under local anesthesia. Postoperative medications were prescribed to the patients for five days. One week following the surgery, the surgical area was examined thoroughly for any postoperative complication related to healing. Patients in both groups were recalled after 1 month, 3 months and 6 months post-treatment. At each visit, oral hygiene instructions were re-enforced and oral prophylaxis was done whenever necessary. Postoperative patient's evaluation was done clinically and radiographically at 3 months and 6 months.

To analyze the post-treatment effect, a paired *t*-test was performed at 14 degrees of freedom and at 95% confidence level. The level of significance was determined by the p value < 0.05.

Table 1

Mean changes in clinical and radiographic SCTA (C-SCTA and R-SCTA, respectively) after SRP

C-SCTA (mm)					R-SCTA (mm)			
Pair of measurement time	Mean difference ± SD	*t*-value	*p*	Remark	Pair of measurement time	Mean difference ± SD	*p*	Remark
Baseline and 3 month	0.725 ± 0.255	10.99	0.00	Significant	Baseline and 3 month	0.725 ± 0.255	0.00	Significant
Baseline and 6 months	0.785 ± 0.288	10.55	0.00	Significant	0.711 ± 0.311	8.83	0.00	Significant
3 month and 6 months	0.059 ± 0.198	1.15	0.26	ns	0.022 ± 0.272	0.31	0.75	ns

SCTA – supracrestal tissue attachment; SRP – scaling and rooth planing; PFS – periodontal flap surgery; SD – standard Deviation; ns – non-significant.

Table 2

Mean changes in total SCTA (C-SCTA + R-SCTA) after SRP

Pair of measurement time	Mean difference (mm)	*t*-value	*p*-value	Remark
Baseline and 3 month	0.743 ± 0.256	11.23	0.00	Significant
Baseline and 6 months	0.774 ± 0.275	10.89	0.00	Significant
3 month and 6 months	0.031 ± 0.229	0.53	0.60	ns

For abbreviations see under Table 1.

Table 3

Mean changes in clinical and radiographic SCTA (C-SCTA and R-SCTA) after PFS

C-SCTA (mm)					R-SCTA (mm)			
Pair of measurement time	Mean difference ± SD	*t*-value	*p*- value	Remark	Mean difference ± SD	*t*-value	*p*- value	Remark
Baseline and 3 month	0.878 ± 0.214	15.88	0.00	Significant	0.780 ± 0.176	17.12	0.00	Significant
Baseline and 6 months	1.068 ± 0.211	19.53	0.00	Significant	0.894 ± 0.241	14.34	0.00	Significant
3 month and 6 months	0.189 ± 0.212	3.46	0.004	Significant	0.114 ± 0.272	1.62	0.12	ns

For abbreviations see under Table 1.

Statistical analysis was done with the help of SPSS (Statistical Package for Social Science) version *13* (SPSS Inc, Chicago, IL, USA).

Results

This study analyzed variations in the SCTA of patients with chronic periodontitis before and after the treatment. SCTA measurements were recorded at baseline and 3 months and 6 months after the treatment. The primary outcome variable of this study was to analyze variations in the SCTA before and after treatment of periodontitis. Following observation were made to meet the objective of the study – variations in C-SCTA after SRP (group 1): the mean C-SCTA at baseline was 2.477 ± 0.429 mm, which increased to 3.202 ± 0.237 mm at 3 months, and further increased to 3.262 ± 0.230 mm at 6 months post-treatment (Table 1); variation in the R-SCTA after SRP (group 1): R-SCTA included the SCTA in mesial and SCTA in the distal region. The mean R-SCTA at baseline was 2.657 ± 0.493 mm, which increased to 3.346 ± 0.330 mm at 3 months, and further increased to 3.368 ± 0.295 mm at 6 months post-treatment (Table 1); variation in T-SCTA after SRP (group 1): T-SCTA at baseline was 2.567 ± 0.452 mm which increased to 3.310 ± 0.293 mm at 3 months which further increased to 3.342 ± 0.267 mm at 6 months post-treatment. The differences in mean values from baseline to 3 months and baseline to 6 months were statistically significant, but they were not statistically significant from 3 months to 6 months post-treatment (Table 2).

Differences in C-SCTA and R-SCTA after SRP (group 1) were: the differences in mean values from baseline to 3 months and baseline to 6 months post-treatment were statistically significant, but they were not statistically significant from 3 months to 6 months post-treatment (Figure 4).

Variations in the C-SCTA after PFS (group 2) were: the mean C-SCTA at baseline was 2.091 ± 0.332 mm, which increased to 2.970 ± 0.206 mm at 3 months, and further increased to 3.159 ± 0.275 mm at 6 months postoperatively (Table 3). Variation in R-SCTA after PFS (group 2) were: the mean R-SCTA at baseline was 2.400 ± 0.249 mm, which increased to 3.180 ± 0.237 mm at 3 months, and further increased to 3.294 ± 0.282 mm at 6 months postoperatively (Table 3).

Variations in the T-SCTA after PFS (gropu 2) were: mean T-SCTA at baseline was 2.245 ± 0.283 mm, which increased to 3.127 ± 0.213 mm at 3 months, and further

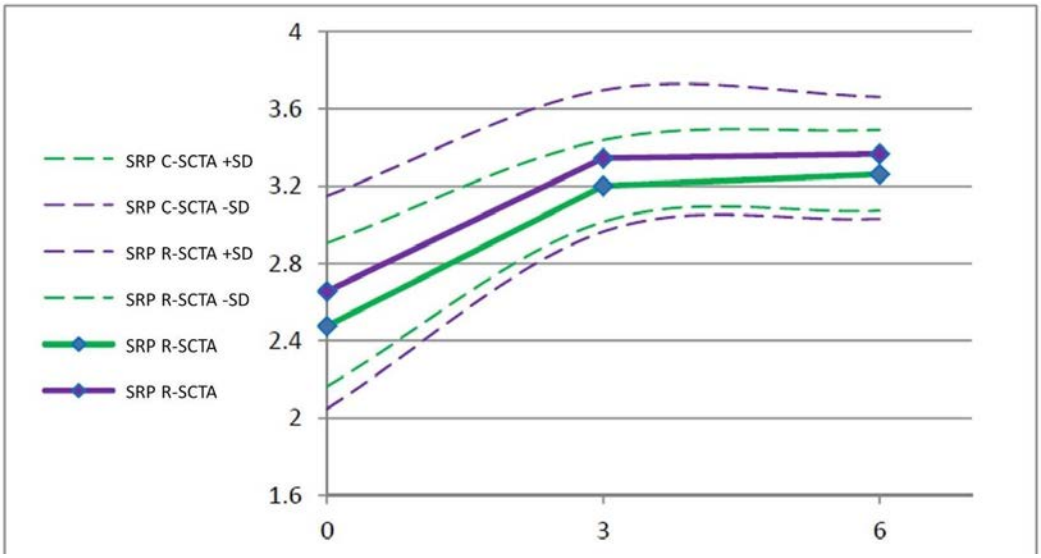

Fig. 4 Comparison between C-SCTA and R-SCTA (in mm) after SRP.
SD – standard deviation; For other abbreviations see under Table 1.

Table 4

Mean changes in total SCTA (C-SCTA + R-SCTA) after PFS

Pear of measurement time	Mean difference (mm)	*t*-value	*p*	Remark
Baseline and 3 month	0.881 ± 0.172	19.75	0.00	Significant
Baseline and 6 months	1.014 ± 0.209	18.76	0.00	Significant
3 month and 6 months	0.132 ± 0.227	2.26	0.04	Significant

For abbreviations see under Table 1.

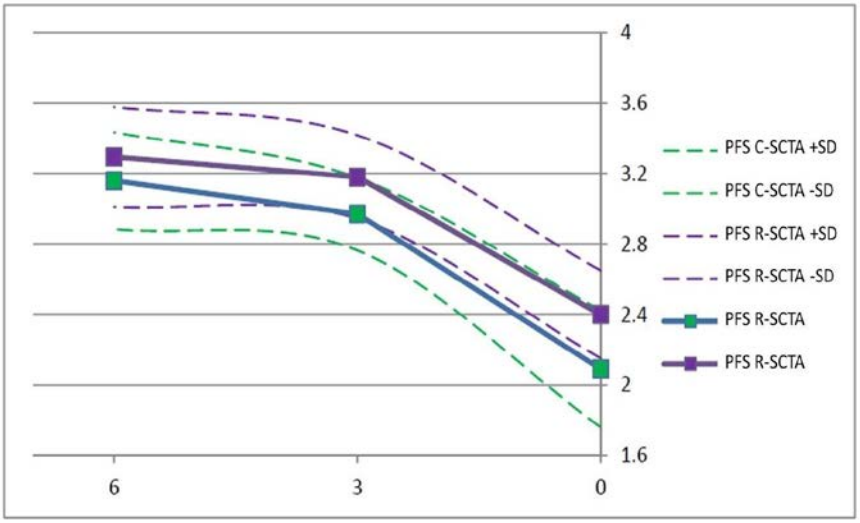

Fig. 5 – Comparison between C-SCTA and RSCTA (in mm) after PFS.
SD – standard deviation; For other abbreviations see under Table 1.

increased to 3.260 ± 0.269 mm at 6 months postoperatively. The differences in mean values from baseline to 3 months, baseline to 6 months, as well as from 3 months to 6 months postoperatively, were statistically significant (Table 4).

Differences in clinical and radiographic SCTA after PFS were: differences in mean values from baseline to 3 months, baseline to 6 months, as well as from 3 months to 6 months postopeartively, were statistically significant (Figure 5).

Comparison between the SCTA of PPD > 3 to < 5 mm treated by SRP and PPD ≥ 5 to 7 mm treated by PFS showed: in the disease, the mean T-SCTA in PPD > 3 to < 5 mm was 2.567 ± 0.452 mm , and 2.245 ± 0.283 mmin PPD ≥ 5 to 7 mm. The difference between the two values was 0.322 mm, which was statistically significant. Three months after the treatment by SRP, the mean T-SCTA was 3.310 ± 0.293 mm and after treatment by PFS the mean SCTA was 3.127 ± 0.213 mm – the difference between the two values (0.183 mm) was not statistically significant. Six months after the treatment by SRP, the mean T-SCTA was 3.342 ± 0.267 mm and after the treatment by PFS it was 3.260 ± 0.269 mm . The difference between the two values

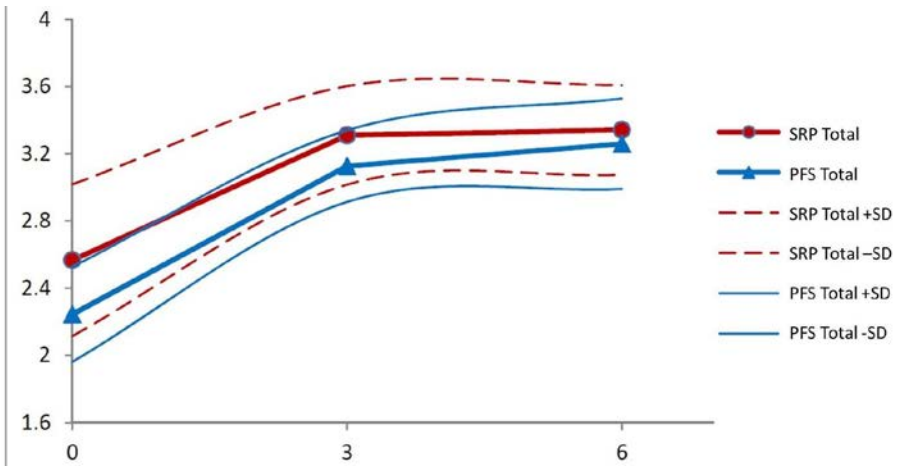

Fig. 6 – Comparison between SCTA (in mm) in probing pocket depth (PPD) 3–5 mm (treated by SRP) and PPD 5–7 mm (treated by PFS).

SD – standard deviation; For other abbreviations see under Table 1.

was 0.082 mm, which was not statistically significant (Figure 6).

Discussion

Periodontal disease is a common inflammatory disease, characterized by periodontal pocket formation and the SCTA loss. Probing depth and clinical attachment loss measurements are routinely recorded at six sites around the tooth, because it is often impossible to anticipate probing depths and loss of attachment from the superficial appearance of the gingiva [15].

After treatment of chronic periodontitis, assessment of the SCTA is essential because there is a frequent need for prosthetic restoration. In addition, it was suggested that SCTA measurements taken from the tissues of a healthy periodontium should not be extrapolated for use in pathologic situations and after periodontal surgery. So, changes in the SCTA may cause failure in the future restoration or prosthesis [16]. Perez et al. [17], in a landmark study, estimated the supra-osseous gingiva (SOG) before and after crown lengthening surgery (CLS). Intra-class correlations were calculated to test for the reliability of transgingival probing (TGP) measurements *versus* direct-bone-level (DBL) measurements. They concluded that TGP is an accurate alternative method to DBL in clinical determining SOG dimensions.

Observations from the study conducted by Goodson et al. [18] revealed that attachment loss precedes radiographic evidence of crestal alveolar bone loss during periods of periodontal disease activity. Sum total of biological width calculated by Gargiulo et al. [5], was 2.04 mm (1.77–2.43 mm). The dimension of the SCTA is known to get affected by tooth type and position, the presence of a restoration, periodontal disease and status after periodontal surgery. The authors finally concluded that there is no fixed dimension of the SCTA.

The value of the SCTA in the health gingiva, as reported in the literature, is 3.39 ± 0.8461. The T-SCTA in the disease, in this study, was found to be 2.406 ± 0.405, with the difference (0.962) that was statistically significant. These results are not in agreement with results of another study in which an average C-SCTA in cases of severe periodontitis was recorded to be 3.95 mm [13]. The resulst from the study revealed that there was a gain in the SCTA after SRP. This may be due to gain in clinical attachment level or formation of long junctional epithelium or both [19]. This process was completed by three months as difference values up to six months were not significant.

In the disease, the mean C-SCTA in the SRP group was 2.477 ± 0.429 mm and the R-SCTA was 2.657 ± 0.493 mm, the difference of which was statistically significant. C-SCTA at baseline was lesser than R-SCTA. It means that buccal, palatal/lingual SCTA at baseline was less than mesial, distal SCTA. Comparison between clinical and radiographic SCTA findings revealed that in the disease the mean C-SCTA in the SRP group was 2.477 ± 0.429 mm and the R-SCTA was 2.657 ± 0.493 mm, the difference of which was statistically significant. These results are very similar to the previous study [20], in which the authors concluded that mesiobuccal and distobuccal SCTA were bigger than mid-lingual or mid-palatal SCTA in the healthy gingiva. After 3 months and 6 months, the clinical and radiographic SCTA findings showed no statistical difference.

Patients in the study group 2 were treated by conventional flap surgery. The difference in mean values from baseline to 3 months and baseline to 6 months postoperatively were statistically significant but from 3 months to 6 months post-treatment were statistically insignificant. Results revealed that there was a gain in the SCTA after PFS. The gain of SCTA was more pronounced in the first 3 months postoperatively, but from 3 months to 6 months the gain in the SCTA was less. This may be due to gain in clinical attachment level or formation of long junctional epithelium or both. This is in agreement with the findings of another study [21], which leads to a concept that tissues of the dento-gingival junction are dynamic rather that static.

Comparison between clinical and radiographic SCTA findings revealed that, at baseline, the C-SCTA was less than R-SCTA. It means that buccal, lingual or palatal SCTA at baseline were less than mesial and distal SCTA. After 3 months, clinical and radiographic SCTA findings showed a statistical difference, but after 6 months,there was no statistical difference.

The periodontal disease progression has an inverse correlation with the dimension of the SCTA. The SCTA regains its original dimensions after periodontal therapy; in cases of shallow pockets (> 3 to > 5 mm) treated with scaling and root planing, it takes 3 months and may take 6 months in moderate pockets (≥ 5 to7 mm) treated by periodontal flap surgery. Most changes in the SCTA occur within first 3 months and remain stable up to 6 months irrespective of the treatment protocol.

Comparison between C-SCTA and R-SCTA showed a significant difference in patients with periodontitis. This difference was not significant after treatment of patients with shallow pockets with scaling and root planing, but was significant in patients with moderate pockets treated by open flap debridement. There was no significant variation in the SCTA of the buccal and palatal/lingual areas, as well as mesial and distal areas of the gingiva.

In the disease, the mean T-SCTA in PPD > 3 to < 5 mm was 2.567 ± 0.452 mm and in the PPD ≥ 5 to 7 mm - it was 2.245 ±0.283 mm. The difference between the two values was 0.322 mm which was statistically significant. This indicates that as the disease progresses, the SCTA reduces. After 3 months and 6 months posttreatment by SRP and PFS, the mean change in the T-SCTA was not statistically significant. In fact, there was more SCTA after SRP than PFS at both, 3 months and 6 months intervals. As about 3 mm of SCTA was established 3 months after PFS, patients treated with Flap and resective osseous surgery for crown lengthening before restorative procedures, could receive the final restoration after 12 weeks postoperatively. Following therapy, the zone of the SCTA appeared to be similar in patients with initially deeper pockets (group 2 treated by PFS) or with initially shallower sites (group 1 treated by SRP).

Conclusion

The periodontal disease progression has an inverse correlation with dimensions of the SCTA. The SCTA regains its original dimensions after periodontal therapy; in cases of shallow pockets (> 3 to > 5 mm) treated with scaling and root planing, it takes 3 months and may take 6 months in moderate pockets (≥ 5 to7 mm) treated by periodontal flap surgery. Most changes in the SCTA occur within first 3 months and remain stable up to 6 months irrespective of the treatment protocol.

Comparison between C-SCTA and R-SCTA shows a significant difference in patients with periodontitis. This difference is not significant after treatment of patients with shallow pockets with scaling and root planing, but shows a significant difference in patients with moderate pockets treated by open flap debridement. There is no significant variation in the SCTA of the buccal and palatal/lingual areas, as well as mesial and distal areas of the gingiva.

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Conflict of interest statement

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Work-related stress among primary healthcare workers

Stres na radnom mestu kod medicinskog osoblja u primarnoj zdravstvenoj zaštiti

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Abstract

Background/Aim. There are data on an increased stress level in primary health care workers. The aim of this paper was to investigate the state of stress among employees in the field of primary health care in order to identify the factors that most affect stress and groups that are particularly susceptible to stress. **Methods.** The study was conducted using a sample of 95 health care workers in the field of primary health care. Data were collected through an anonymous survey consisting of two parts. The first part of the survey included questions related to the characteristics of the workplace and professional and socio-demographic characteristics of the employees. The second part of the survey was based on the Behavioral Health Concepts (BHC) stress test, which is used to estimate the adaptation to stress across four dimensions: overall assessment, quality of life assessment, symptomatology, and level of functioning. **Results.** Although the total number of respondents fell under the group of moderate stress (mean = 3.97), using descriptive statistical analysis, it was discovered that, 4.2% of the respondents had an increased level of stress. The variance analysis demonstrated that there were statistically significant differences ($p < 0.00$) between the effects of educational variables (F = 11.68), workplace (F = 14.07) and work time (F = 9.16) on overall stress. Significant interaction between variables workplace and work time was also found [F (2.72) = 3.22; $p < 0.046$]. **Conclusion.** Primary health care employees have an increased level of stress, which depends on both the working conditions and the personal characteristics of the employees.

Key words:
primary health care; health personnel; stress, psychological; risk factors; surveys and questionnaires; serbia.

Apstrakt

Uvod/Cilj. Postoje podaci o povišenom nivou stresa kod radnika u primarnoj zdravstvenoj zaštiti. Cilj ovog rada je bio da se istraži stanje stresa kod zaposlenih u oblasti primarne zdravstvene zaštite radi identifikacije faktora koji najviše utiču na stres, kao i identifikacije grupa koje su posebno podložne stresu. **Metode.** Istraživanje je sprovedeno na uzorku od 95 zdravstvenih radnika zaposlenih u oblasti primarne zdravstvene zaštite. Podaci su prikupljeni pomoću anonimne ankete koja se sastojala iz dva dela. Prvi deo ankete je sadržao pitanja koja se odnose na karakteristike radnog mesta i profesionalne i socio-demografske karakteristike zaposlenog. Drugi deo ankete je bio baziran na *Behavioral Health Concepts* (BHC) stres testu, koji se koristi za procenu adaptacije na stres preko četiri dimenzije: ukupna ocena, ocena kvaliteta života, simptomatologija i nivo funkcionisanja. **Rezultati.** Iako su ispitanici pripadali grupi umerenog nivoa stresa (srednja vrednost = 3,97), upotrebom deskriptivne statističke analize utvrđeno je da je kod 4,2% ispitanika postojao povišen nivo stresa. Analizom varijanse ustanovljeno je da postoje statistički značajne razlike ($p < 0.00$) između uticaja varijabli obrazovanja (F = 11.68), radnog mesta (F = 14.07) i radnog staža (F = 9.16) na ukupan stres. Takođe, pronađena je i značajna interakcija između varijabli radno mesto i radni staž [F(2,72) = 3.22; $p < 0.046$]. **Zaključak**. Zaposleni u primarnoj zdravstvenoj zaštiti imaju povišen nivo stresa, što zavisi kako od uslova rada, tako i od ličnih karakteristika zaposlenih.

Ključne reči:
zdravstvena zaštita, primarna; zdravstveno osoblje; stres, psihički; faktori rizika; ankete i upitnici; srbija.

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Introduction

So far, the published analyses of working in outpatient units in Serbia and the City of Belgrade have suggested that there is an increased workload of medical staff employed in primary health care in relation to the statutory scope of the provision of health services [1]. These data suggest that primary health care workers are exposed to stress at the workplace.

There are many consequences from chronic stress. In an individual under stress, the psychological consequences such as high levels of irritability, frustration, anxiety, aggression, nervousness, apathy, depression, disorientation, loss of self-esteem, as well as somatic consequences, such as high blood pressure, arrhythmia, and difficult breathing are the most striking. If these problems persist for a long time, they can lead to serious disorders of the digestive system, cardiovascular system, immune system, locomotor system resulting in atherosclerotic changes in the blood vessels, digestive disorders, frequent colds, malignant diseases, asthma and other long-term diseases [2–7]. Regardless of the resistance of psychic and physical constitutions to the effects of stressors, high levels of stress as well as chronic stress have a negative impact on performance [8–10].

In the literature dealing with modern theories of stress at the workplace, the integrative-process concept has been singled out as the most dominant [11]. This model takes into account external stressors, which primarily concern characteristics of work, workplace, work process and management, working atmospheres [12–13] and dispositional characteristics of an individual, of which stress resistance, the speed of overcoming stress and stress sediment are particularly significant [14]. One of the variants of this concept is the "effort-reward" model, based on the premise that breaking the reciprocity between effort invested at work and material compensation is the main cause of emotional and later health problems among employees [15]. This model can be largely applied to the analysis of stress among employees in the health sector [16]. As studies show, doctors in the United States are not satisfied with their work. Namely, as many as 78% of the respondents stated that they did not enjoy their work or that they found much less fulfillment at work than at the beginning of their career, while 68% of the respondents would not recommend medicine as a professional orientation [17].

In the medical profession, in addition to the usual stress factors at the workplace, there are specific causes of stress, such as acute conditions requiring urgent intervention, constant contact with death, serious illness or persons with physical disabilities, and an unpleasant feeling due to the inability to provide adequate assistance to a patient. It can be assumed that the risk of professional omissions and iatrogenic defects that can have drastic consequences for the health and life of patients constitutes an additional burden for health workers, especially in developing countries, where material factors and a lack of resources have a significant outcome for treatment.

Primary health care as a pillar for prevention and preservation of the nation health is an especially important part of the health system through which the functioning of state organs and the overall situation in a society is reflected. Factors that can contribute to stress among employees in the field of primary health care are, in addition to inadequate material-technical conditions for work, an insufficient number of employed professionals, long waiting periods, a demanding administration and short amount of time available for the doctor, the availability of time for a patient check-up, as well as the differences in material compensation for work in the private and public health sector [18].

In accordance with the above-mentioned, some empirical studies have confirmed that there is an increased level of stress at workplace among medical personnel in Serbia and the risk of burnout syndrome [16], and that it could be prevented, which requires more detailed research and identification of groups that are especially susceptible/exposed to stress. Personal characteristics such as gender, age, education, workplace, family and social status are potential indicators that act upon on the vulnerability of an individual in stressful situations [7], and the relationship of these indicators with the level of stress among employees in health care institutions in Serbia has not yet been thoroughly examined.

The aim of this paper was to investigate the state of stress among employees in the field of primary health care in order to identify factors that most affect stress and groups that are particularly susceptible to stress.

Methods

For the purpose of this research, the results of the survey from one of the total of 16 health centers in the territory of the City of Belgrade were analyzed (in order to protect the anonymity of the respondents, the name of the institution in which the survey had been conducted was omitted). The selected institution can be considered representative because of a number of branches in urban and suburban areas. The study was conducted on a sample of 95 subjects, which included 10 men and 85 women, the average age being 43 years. The ratio of male and female respondents was proportional to the gender representation among the employees at the selected institution where the research was conducted [16, 18]. The sample size was selected after analyzing the statistical power for the analysis of variance for the draft 3 × 2 (three levels of factor A with a combination of two levels of factor B at each level of factor A), using $\alpha = 0.05$, the strength $f = 0.80$, and for the mean expected effect $\delta = 0.4$ [19, 20]. In addition to the above assumptions, the minimum sample size should be 75 examinees. When forming the sample, the structure of subunits was balanced by variables related to the workplace (workplace and department) and by intervening variables (gender, marital status, number of children, average daily number of examinations, years of work experience). Of the above variables, in terms of statistical power analysis under factor A, years of work experience from three levels (up to 15 years, from 15 to 25 years, and over 25 years of service) were considered, while the variable workplace and other in-

tervening variables that had two levels represented factor B in individual analysis of variance.

All respondents were asked to solve an anonymous questionnaire consisting of two parts. The first part of the questionnaire consisted of questions relating to sex, education, marital status, number of children, workplace, department in which the employee performs their duties, average daily number of patients, and years of work experience. The second part of the questionnaire was based on the Behavioral Health Concepts (BHC) stress test, which is a standardized questionnaire designed using a factor analysis that reduced the initial inventory from about 400 to 27, best describing three factors: quality of life, level of functioning, and symptomatology. The assessment of quality of life consists of four sub-factors: autonomy, self-confidence, social support, and physical health. The assessment of symptomatology is described by three sub-factors: depression, somatization, and paranoia. The level of functioning in everyday life relates to issues related to misconduct (physical and verbal conflicts) and the level of social skills development (in both business and private life). The result of the BHC test is a stress barrier, ranging from 0 to 5 for each of the sub-factors, as well as for total stress. A lower numerical value describes a higher degree of stress and *vice versa*, a greater number represents a better adaptation to stress. Depending on the results, respondents were further classified into one of four categories: well-adapted, moderately shaken, highly stressed, and extremely stressed, for each of the above-mentioned dimensions. The BHC stress test has good psychometric characteristics [21], which were confirmed in Serbia through "various research with teachers in elementary and secondary school, university students, nurses, and also in Serbian post and in some sectors of Serbian army" [22].

All respondents agreed to participate in the research voluntarily. They were in advance informed about their tasks both orally and in writing. Also, they were informed that their personal data would be secret and that they would be able to leave the study at any time without any consequences. The research was approved by the Dean of the Faculty of Physical Culture and Sports Management, and in accordance with the Code of Professional Ethics of the Singidunum University, as well as with the Ethical Principles and the Code prescribed by the American Psychological Association (APA). The quantified data was processed by descriptive statistical analysis, *t*-test, hi-square (χ^2) test and analysis of variance using the software package SPSS version 22.0.

Results

The results of descriptive statistical analysis (Table 1) showed that the average respondent was under moderate stress (3.97). Also, the average respondent was characterized by subclases the quality of life (3.92) and symptomology (3.81) as moderately stressed, while on the subscale functionality he/she was assessed as well adapted to stress (4.20). An analysis of the percentage representation of stress categories (Table 2) showed that, according to total stress, 4.2% of the total sample belonged to the group of elevated stress, while on subscales the quality of life and symptomatology these values were significantly higher (11.6% and 14.7%, respectively), and on the subscale symptomatology, phenomenon of extremely stressed workers was also recorded (1.1%).

Analyzing variables that affect the occurrence of stress, it could be concluded that men on all three subscales, as a total stress rating, fell under the category of good adaptation, while women were in a group with moderate stress, excepting the subscale of functionality, where they also belonged to a group of moderate stress. Employees over 40 years of age achieved lower results on total stress and subscales compared to employees under 40 years of age. In accordance with this, the results obtained from the variable work experience showed that the group of workers over 25 years of age was the most at risk for stress, while the best adaptation had the group of employees with working experience ranging from 15 to 25 years. There were no significant differences in adaptation to stress among employees who had not children or had only one child in relation to employees with two or more children, except for the symptomatology subscale, according to which childless workers or those with one child were better adapted, although this difference was not statistically significant. Unmarried health care workers belonged to a well-adapted group, unlike their married colleagues who were under moderate stress, with the exception of the functionality subscale. Similarly, highly educated employees belonged to the group of those who were well-adapted to stress, as opposed to workers who had completed high school and belonged to the group with moderate stress. These differences were also noticeable in terms of the variable employment, which very similarly differentiated the sample to the subunits, as well as the variable education. With the exception of two respondents, all subjects with higher education were employed as doctors. Differences in stress levels among employees of different educational levels were even more pronounced when the sample was segmented according to the variable employment. According to the percentage distribution of stress categories (Table 2), there were differences in the adaptation to stress among physicians and nurses and technicians. Namely, it was noticeable that doctors in all subscales were better adapted to stress than other members of medical staff, and this figure was most noticeable when considering total stress – none doctors and 7.3% of the nurses and technicians belonged to the category of employees under increased stress. According to the department in which they work and the average daily number of patients, there was little difference among respondents in stress adaptation. The exception was the variable total stress, according to which employees who had up to 40 patients per day belonged to the group with good adaptation, while the employees who see more than that number of patients fell under the group of employees with moderate stress (3.97).

Although all the average values suggested moderate stress and good adaptation of medical staff, the number of minimal scores (Table 1) and the percentage representation

of stress level categories (Table 2) indicated that among employees there were those who belonged to the group of high and extreme stressed in the total sample as well as in subunits.

relation to the work experience, i.e. the length of health care service. There was are statistically significant difference ($p < 0.05$) between respondents with different marital status regarding to the quality of life, and between males and

Table 1

Descriptive statistical analysis and variance analysis of stress among community health centre workers – complete sample and subsamples

Parameters	Total stress						Life quality			Symptomatology			Functionality		
	n	min	max	M	SD	*p**	M	SD	*p**	M	SD	*p**	M	SD	*p**
All patients	95	2.70	4.96	3.97	0.56		3.92	0.64		3.81	0.82		4.20	0.50	
Gender															
m	10	3.43	4.93	4.28	0.62	0.07	4.20	0.79	0.15	4.28	0.85	0.05	4.38	0.50	0.24
f	85	2.70	4.96	3.94	0.54		3.88	0.62		3.75	0.81		4.18	0.51	
Age, (years)															
under 40	40	3.05	4.96	4.04	0.51	0.32	4.03	0.51	0.16	3.84	0.74	0.75	4.26	0.54	0.33
over 40	55	2.70	4.93	3.93	0.59		3.84	0.60		3.78	0.89		4.16	0.48	
Number of children															
1	49	2.70	4.93	3.99	0.56	0.81	3.93	0.66	0.90	3.85	0.81	0.59	4.19	0.47	0.84
> 1	46	2.86	4.96	3.96	0.57		3.91	0.63		3.76	0.84		4.21	0.55	
MS															
single	28	3.13	4.96	4.10	0.48	0.14	4.11	0.56	0.05	4.02	0.67	0.11	4.18	0.53	0.80
married	67	2.70	4.93	3.92	0.58		3.84	0.66		3.72	0.87		4.21	0.50	
Education															
secondary	53	2.70	4.93	3.81	0.54	0.00	3.72	0.58	0.00	3.58	0.78	0.00	4.13	0.52	0.14
college & faculty	42	3.34	4.96	4.18	0.52		4.17	0.72		4.09	0.88		4.29	0.49	
Position															
doctor	40	3.49	4.93	4.21	0.49	0.00	4.20	0.47	0.00	4.14	0.79	0.00	4.30	0.52	0.12
nurse	55	2.70	4.96	3.80	0.55		3.71	0.68		3.56	0.77		4.13	0.49	
LS (years)															
< 15	36	3.05	4.96	4.10	0.51		4.04	0.55		3.98	0.76		4.28	0.55	
15–25	29	3.13	4.93	4.16	0.50	0.00	4.03	0.61	0.03	4.09	0.67	0.00	4.37	0.43	0.00
> 25	30	2.70	4.90	3.64	0.53		3.66	0.72		3.32	0.83		3.95	0.43	
Unit of health care															
adults	54	3.05	4.90	3.99	0.54	0.72	3.90	0.58	0.81	3.86	0.78	0.45	4.21	0.52	0.83
children	41	2.70	4.96	3.95	0.59		3.93	0.72		3.73	0.88		4.19	0.49	
Number of patients /day															
< 40	46	2.70	4.96	4.01	0.59	0.53	3.94	0.68	0.78	3.90	0.88	0.31	4.20	0.53	0.99
> 40	49	2.86	4.90	3.94	0.53		3.90	0.61		3.72	0.76		4.20	0.49	

n – number of respondents; min – minimum; max – maximum; M – mean; SD – standard deviation; *One – way ANOVA; m – male; f – female; MS – maritial status; LS – length of service.

Table 2

Stress level categories among health care workers (in percentages)

Health care workers	Total stress				Life quality				Symptomatology				Functionality			
	WA	MS	IS	ES	WA	MS	IS	ES	WA	MS	IS	ES	WA	MS	IS	ES
All	53.7	42.1	4.2	0.0	57.9	30.5	11.6	0.0	44.2	40.0	14.7	1.1	70.5	28.4	1.1	0.0
Position																
doctor	70.0	30.0	0.0	0.0	75.0	22.5	2.5	0.0	65.0	27.5	2.5	0.0	77.5	22.5	0.0	0.0
nurse	41.8	50.9	7.3	0.0	45.5	36.4	18.2	0.0	29.1	49.1	20.0	1.8	65.5	32.7	1.8	0.0

WA – well-adjusted; MS – moderate stress; IS – increased stress; ES – extreme stress.

The results of the analysis of variance showed that there were statistically significant differences in total stress as well as in subclases the quality of life and symptomatology between health care workers with different education status and, accordingly, between doctors and nurses ($p < 0.00$). Similarly, there were statistically significant differences in total stress rate, as well as in subclasses the quality of life, symptomatology and functionality among respondents in

females regard in to subclass symptomatology. On the edge of statistical significance ($p < 0.07$), there was difference between male and female respondents regarding to variable total stress (Table 1).

In addition to the effects of individual workplace dimensions on stress variables, their interactions were also examined. In the case of total stress, a significant interaction was observed between the workplace and work experience [F

(2.72) = 3.22; $p < 0.046$]. The total stress of doctors and nurses varied in different ways from years of service (Figure 1). The interaction of these two factors could be explained to a large extent by their interaction with the quality of life [F (2.72) = 6.37; $p < 0.003$].

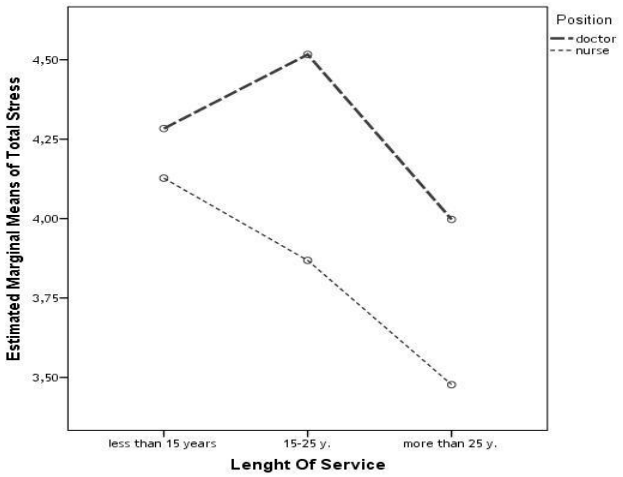

Fig. 1 – Differences in the average values of total stress among nurses and doctors by length of service.

Regarding the functionality, a significant interaction was observed between factors: work position (doctors vs. nurses) and workplace (adult health care department vs. children health care department) [F (1.72) = 10.60; $p < 0.002$], and the factor division and working time [F (2.72) = 3.36; $p < 0.040$]. The functionalities of doctors and nurses working in the adult health care department and the children health care department varied in different ways, while the functionality of employees in the adult health care department varied in different ways in relation to the years of service (Figure 2–4).

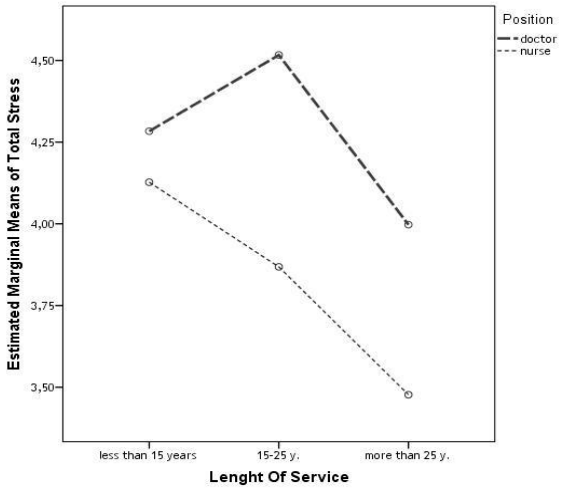

Fig.2 – Differences in the average values of the life quality of nurses and doctors by length of service.

In relation to the quality of life, a significant interaction of factors working hours and number of examinations was obtained [F (2.72) = 4.25; $p < .018$]. The quality of life varied in different ways for employees who had up to 40 patients per day and those had over 40 patients per day compared to years of their service (Figure 5).

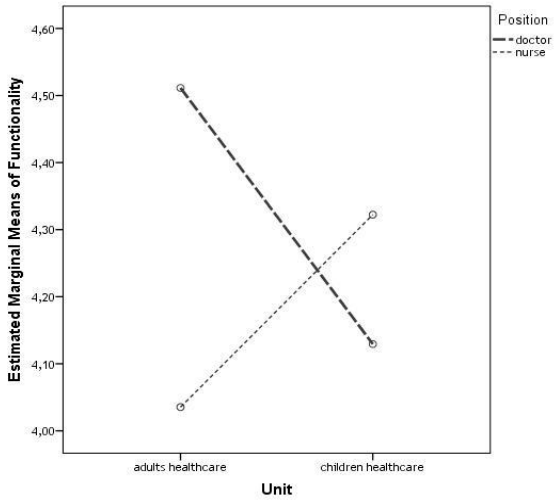

Fig. 3 – Differences in the average values of the functionality of nurses and doctors by units.

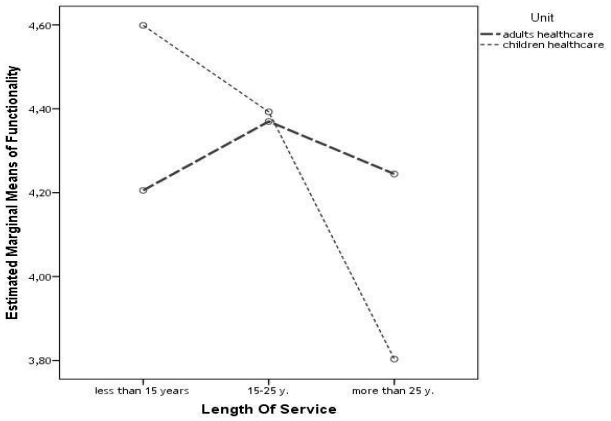

Fig. 4 – Differences in the average values of functionality of health care workers working with children and adults by length of service.

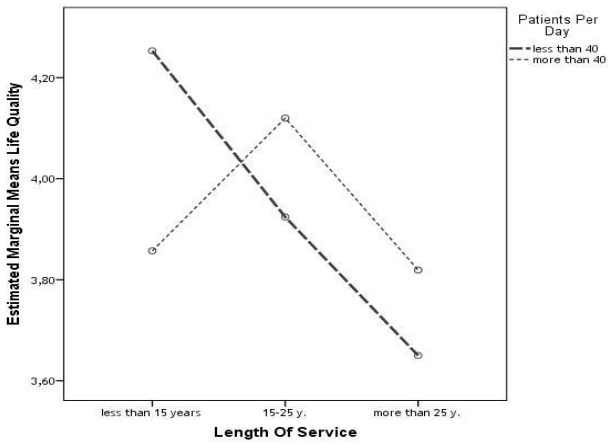

Fig. 5 – Differences in life quality average values of those who examine less than 40 patients per day and those examine more than 40 patients per day by length of service.

Discussion

The results of the survey showed that employees in the field of primary health care, though in the long term exposed to stress, were better adapted to it than could be expected given the specificity of their work, working conditions, insufficient number of employees, large volume of the work and material compensation. These facts are important because stress in the workplace can lead to a number of psychophysical problems in the workforce, a decline in work capacity, and concentration levels that can cause failures in work and fatal consequences. Although in a small percentage, the presence of extremely stressed workers is also cause for emergency intervention measures in order to prevent damage to their health, as well as consequences for users of health system services.

Most of the results obtained in our study, concerning stress levels and its relationship to personal characteristics and working conditions, are in line with theoretical frameworks and findings of previous empirical studies [23–28]. The greatest impact on stress adaptation is related to years of working hours and employment, or education. According to the results of the research, as the level of the respondents' education grows, their adaptation to stress also grows, so the best educated respondents are the best adapted. Since doctors are mainly amongst those in the highly educated group, while nurses and technicians have a middle degree of professional education, it is not surprising that by comparing these two groups, the results are almost identical as when respondents compare in relation to their education levels. Similarly, it can be noticed that when comparing the sample segments according to economic status – nurses and technicians have lower incomes, those with lower incomes are less adapted to stress. The discovery that nurses demonstrate more symptoms of burning out at work was earlier shown in other studies [23–26].

When looking at the interaction of the factors, the work experience and workplace, it can be seen that, unlike nurses and technicians whose adaptation to stress constantly decreased with length of service, in the case of doctors employed 15–25 years at the same workplace, adaptation to stress was better in relation to their colleagues with shorter and longer working time. This finding may be a supplement to the explanation of the above-mentioned findings of empirical studies [23–26]. Namely, it can be assumed that with the passing of time, doctors gain confidence in themselves and their skills, which makes them easier to deal with stress at the workplace, while the decline in adaptation after 25 years of service could be in part attributed to aging and consequently weakening of biological, emotional and mental capacities to combat stress, as well as the emergence of monotony in the workplace and the decline in skills and motivation for further training. Bearing in mind that there are no statistically significant differences between employees older than and under 40 years, it is more likely that this finding is not a consequence of aging or other reasons, but possible due to an insufficient sample size, and an especially insufficient number of young and extremely old respondents. This finding must be taken with reserve, and may be the reason for more detailed future research.

Although there were no statistically significant differences between medical staff employed in adult and child health care departments, the interaction between the employment factor and the department speaks about the nature and organization of work as the primary cause of stress in primary health care. Nurses and technicians, when considering the overall pattern, are less adapted to stress than physicians when considering the sample from the adult health department. However, in the children health care department, medical nurses and technicians were slightly better adapted to stress than doctors This finding can be explained by the fact that medical work is more complex and responsible when dealing with children, while the pressure of patients on nurses and technicians as a result of greater fluctuation of patients is more pronounced in the adult healthcare department. It was demonstrated that the health care department for adults had a positive effect on work experience, so that those that the best adapted to stress were workers with 15–25 years of service. On the other hand, the length of service in the child healthcare department had a negative effect on adaptation to stress, and long-term employees in this department were a vulnerable group, which speaks in favor of the previous assumptions about the nature of work as the main source of stress in this study. These findings could be a complement to theoretical explanations of the relationship between workplace characteristics and stress [24, 27, 28], in which an emphasis is placed on interpersonal relationships and management attitude as the key characteristics of the workplace responsible for stress of employees.

It is somewhat surprising finding that, although there was a statistically significant link between daily number of examinations and the level of stress among employees, it had relatively small impact on stress. It is somewhat contrary to the findings of previous studies [24]. However, compared to some other countries where the number of daily examinations and interventions of medical staff does not exceed 10, the number of patients per day in this study ranged between 30–40 and over 40 indicating that the load was present to the extent that further increases in the number did not affect stress. Moreover, the interaction of factors, the work hours and the number of examinaions (Figure 5) shows that a large number of examinations mostly had a negative impact on adaptation to stress of employees who had a relatively short work experience (up to 15 years). Accordingly, doctors who had a large number of examinations and interventions per day were better adapted than those who were employed for more than 30 years, which indicates that with experience they gain skills and build a relationship that makes these doctors more demanding than others, and satisfaction at workplace represents a significant source of adaptation to stress. This finding is in accordance with the empirical findings that the assessment of one's own competence is cross-sectioned with high workload results in a burnout [24].

Previous research on this topic has demonstrated that women adapt less to stress at work [23, 26, 28], which can be attributed to the interdependence of psychological and biological specificity of the female sex, the use of varying coping strategies [28] or the inability to balance professional and family obligations. The results of this study argued in favor of the third assumption, but due to small number of males surveyed, they can only be taken as the starting point for future research. Although they are not unambiguous, the results presented also support the fact that in primary health care, the work organization is often more stressful than the nature of work, and that there are aspects of the work organization that the management of the institution could influence in order to reduce stress among employees, which is in line with the findings of previous studies [24, 26, 28].

The major limitations of this study are that all potential factors that could affect the occurrence of stress in the workplace have not been covered. During the research, it was noted that the inventory of independent and intervening variables was insufficient, and it should be expanded with indicators of both personal and material characteristics of the employees, as well as the social and material working conditions, which would require additional increases of the sample size. In order to better understand the state of stress of medical staff in primary health care, it is necessary to conduct similar research on a larger sample of examinees, and with an expanded inventory of independent and intervening variables.

Conclusion

It can be concluded that a certain number of employees in the field of primary health care have an increased level of stress, which is primarily related to the conditions and nature of the work, but also to personal and family characteristics. The results of this study show that university-educated men with 15 to 25 years of service are those best adapted to stress.

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Development of one-year major adverse cardiac events risk index in patients with acute coronary syndrome and diabetes mellitus who underwent percutaneous coronary intervention

Razvoj indeksa rizika od velikih vaskularnih događaja tokom godinu dana posle perkutane koronarne intervencije kod bolesnika sa akutnim koronarnim sindromom i dijabetesom melitusom

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Abstract

Background/Aim. Patients with acute coronary syndrome (ACS) and diabetes mellitus (DM) have an increased risk of major adverse cardiovascular events (MACE) after percutaneous coronary intervention (PCI), which is not estimated sufficiently-multidimensionally in terms of type and severity of the ACS and/or DM and angiographic findings. The study was intended to validate and develop an index of metabolic, angiographic, anatomic and clinical risk factors for one-year MACE after conducted PCI in patients with ACS and DM. **Methods.** A prospective cross-sectional study was performed in patients with DM and ACS. In the PCI period the following risk factors were recorded: 1) age and metabolic variables – glycosylated hemoglobin (HbA1c), total cholesterol, and triglycerides levels in the blood; 2) endocrinological variables – DM therapy and type of DM; 3) ACS modality; 4) radiological/anatomical variable – SYNTAX score, and 5) clinical variables in modified age, creatinine, ejection fraction (ACEF) score. One-year MACE were recorded. **Results.** From a total of 136 consecutive patients, 55 of them developed at least one MACE in one-year follow-up. A high predictive risk index was evaluated that assessed particular or associated risks for one-year MACE (c statistic = 0.879) in the study population, defined by: SYNTAX score > 21, modified ACEF score > 1.38, HbA1c ≥ 8%, triglyceridemia ≥ 2.3 mmol/L in patients with insulin therapy, and ACS modality – unstable angina pectoris. The constructed risk index for one-year MACE (MACERI) had better predictive characteristics than SYNTAX score (c statistic = 0.798), as well as ACF score (c statistic = 0.744). **Conclusion.** MACERI can potentially have great application in future risk factors studies for one-year MACE in patients with DM and ACS who underwent PCI, because with it the effects of these factors are measured multidimensionally at valid and accurate manner.

Key words:
coronary artery disease; coronary angiography; diabetes mellitus; comorbidity; cardiovascular diseases; acute disease; risk factors.

Apstrakt

Uvod/Cilj. Bolesnici sa akutnim koronarnim sindromom (AKS) i dijabetes melitusom (DM) imaju povećan rizik od pojave velikih neželjenih kardiovaskularnih događaja (VNKD) nakon perkutane koronarne intervencije (PKI), ali on je nedovoljno višedimenzionalno procenjen u odnosu na vrstu i težinu AKS i/ili DM i angiografske nalaze. Ova studija je imala za cilj da validira i razvije indeks metaboličkih, angiografsko-anatomskih i kliničkih faktora rizika za VNKD u toku jedne godine posle sprovedene PKI kod bolesnika sa AKS i DM. **Metode.** Sprovedena je prospektivna studija preseka kod bolesnika sa DM i AKS, kojima su u periodu PKI evidentirani sledeći faktori rizika: 1) metaboličke varijable – nivoi glikoziliranog hemoglobina (HbA1c), ukupnog holesterola i triglicerida u krvi; 2) endokrinološke varijable – terapija DM i tip DM; 3) modalitete AKS; 4) radiološke/anatomske varijable – SYNTAX skor i 5) kliničke varijable u modifikovanom starost, kreatinin, ejekciona funkcija (engl. *age, creatinine, ejection fraction* – ACEF) skoru. VNKD su evidentirani do godinu dana posle PKI. **Rezultati.** Nakon PKI, od konsekutivno uključenih 136 bolesnika, njih 55 razvilo je bar jedan VNKD u periodu praćenja od jedne godine. Konstruisan je visoko prediktivni indeks rizika kojim su procenjeni zasebni ili združeni rizici

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od VNKD (c statistika = 0,879) u studijskoj populaciji, a koji su definisani SYNTAX skorom > 21, modifikovanim ACEF skorom > 1,38, HbA1c ≥ 8%, trigliceridemijom ≥ 2,3 mmol/L kod bolesnika na insulinskoj terapiji, kao i modalitetom ACS – nestabilna angina pektoris. Konstruisani indeks rizika (IR) od VNKD (IRVNKD) imao je bolje prediktivne karakteristike u odnosu na SYNTAX skor (c statistika = 0,798), kao i ACEF skor. **Zaključak.** IR-VNKD potencijalno može da ima veliku primenu u budućim istraživanjima faktora rizika od VNKD koji nastaju do jedne godine posle PKI kod bolesnika sa DM i AKS, jer se njime učinci ovih faktora višedimenzionalno validno i precizno mere.

Ključne reči:
koronarna bolest; angiografija koronarnih arterija; dijabetes melitus; komorbiditet; kardiovaskularne bolesti; akutna bolest; faktori rizika.

Introduction

Diabetes mellitus (DM) is the most important risk factor for coronary artery disease (CAD) and stroke [1]. Patients with DM have higher risk of CAD than non-diabetic patients [2]. More than 80% of all lethal outcomes in patients with DM are caused by atherosclerosis [1]. These patients more frequently have severe CAD and mortality from stroke than non-diabetic patients [1]. Having in mind their poor prognosis, in these patients the choice of the best CAD treatment (lifestyle correction, pharmacological therapy, revascularization, surgery) is of crucial importance [3]. Patients with severe CAD and DM often have multivessel disease and they are more frequently candidates for coronary artery bypass graft (CABG) surgery than non-diabetic patients [4, 5]. On the other hand, revascularization with the use of invasive percutaneous coronary intervention (PCI) is a more recent method in medicine due to its effectiveness in removing the most severe outcomes of coronary ischemia [6]. In order to closely monitor the treatment effects in patients with CAD and DM, and to improve medical decision-making in the choice between different alternatives and precise predictions related to the most important therapeutic outcome such as major adverse cardiac events (MACE), there was a need to develop unique angiographic and/or clinical instruments for CAD complexity measurement.

The SYNTAX score is an angiographic grading tool for determining the complexity of CAD in patients undergoing revascularization in combination with angiographic classifications aiming to grade the coronary anatomy with respect to the number of lesions, their location and functional impact of angiographically obstructive lesions [7–11]. In the SYNTAX trial after various longer follow ups, different "cut points" for the SYNTAX score are defined, which determine the level of risk for the primary outcome of CAD treatment. Thus, it is defined that patients with the SYNTAX score ≤ 22 have less complex CAD and better treatment outcomes [12–14]. The SYNTAX score has predictive value in different clinical settings including acute coronary syndrome (ACS) [7, 11, 15–21].

Modification of the original Age, creatinine, ejection fraction (ACEF) score [22] has recently been presented. The original ACEF score uses age, serum creatinine and ejection fraction of the left ventricle for adverse cardiovascular events prognosis in patients with DM and ACS. The modified ACEF score, uses creatinine clearance (CrCl) instead of serum creatinine providing a better assessment of renal function and improving predictive value in cardiovascular risk assessment, especially with regard to the prediction of the MACE development [23, 24].

However, there are no reports of the development of a predictive tool, which would combine the assessment of anatomical, clinical and metabolic risk factors for MACE in patients with ACS and DM. The aim of this study was assessment of the adequacy and criterion validity of a risk index which combines the SYNTAX score, the modified ACEF score and the metabolic risk factors for one-year MACE in patients with ACS and DM who underwent PCI.

Methods

Study design

The study was designed as a prospective, cross-sectional study in patients with ACS and DM who underwent PCI and who were monitored by one-year MACE. It was conducted in the period 2012–2014 at the Department of Cardiology and Invasive Cardiology, General Hospital Valjevo, Valjevo, Serbia. The study was approved by the local Ethics Committee and all the patients signed informed consent form.

Patients

The study population included patients who underwent PCI, men and women, older than 18, with ACS and DM. Patients had diagnosis of DM for at least one year according to current guidelines. ACS was defined as: acute myocardial infarction with ST elevation (STEMI) in the patient with characteristic symptoms of myocardial ischemia lasting for more than 20 min in association with persistent electrocardiographic ST elevation (STE) of more than 1 mm (0.1 mV) in two or more contiguous leads, or new, or presumed new left bundle branch block and subsequent release of biomarkers of myocardial necrosis; acute myocardial infarction without electrocardiographic ST elevation (NSTEMI) in the patients with characteristic symptoms of acute chest pain lasting for more than 20 min accompanied by ST depression of more than 1 mm (0.1 mV) and T wave inversion in two or more contiguous leads, with positive biomarkers of myocardial necrosis and unstable angina pectoris (UAP) in the patients with characteristic

Table 1

Description of continuous variables with the significance of the difference between MACE patients' groups

Variables	All patients (n = 136) mean ± SD	Patients with MACE		
		No (n = 81) mean ± SD	Yes (n = 55) mean ± SD	*p* (*t* value)
Age (years)	62.51 ± 9.35	60.73 ± 8.72	65.13 ± 9.70	0.007 (-2.758)
Total cholesterol (mmol/L)	5.85 ± 1.28	5.90 ± 1.27	5.78 ± 1.29	0.610 (0.511)
Triglycerides (mmol/L)	2.21 ± 1.27	2.24 ± 1.36	2.17 ± 1.14	0.732 (0.343)
HbA1c (%)	8.07 ± 1.18	7.7 ± 1.14	8.51 ± 1.12	0.000 (-3.726)
Creatinine clearance (mL/min)	9.85 ± 5.08	91.71 ± 4.86	89.58 ± 5.19	0.016 (2.436)
Left ventricular ejection fraction (%)	46.57 ± 8.35	49.52 ± 6.22	42.24 ± 9.22	0.000 (5.120)
SYNTAX score	23.26 ± 10.17	19.28 ± 6.87	29.13 ± 11.39	0.000 (-5.742)
Modified ACEF score	1.41 ± 0.44	1.25 ± 0.28	1.64 ± 0.53	0.000 (-4.975)

SD – standard deviation; HbA1c – glycosylated hemoglobin; MACE – major adverse cardiac events; ACEF – age, creatinine, ejection fraction.

symptoms of acute chest pain lasting for more than 20 min accompanied by electrocardiographic ST depression of more than 1 mm (0.1 mV) and T wave inversion in two or more contiguous leads, with negative biomarkers of myocardial necrosis. Patients with previous CABG or PCI on coronary arteries and patients with cardiogenic shock were not included in the study.

Procedures

According to the primary PCI (pPCI) protocol, only culprit lesion was treated during procedure. pPCI was performed in STEMI patients up to 48 hours from the onset of characteristic symptoms of myocardial ischemia. The decision whether to treat non-culprit lesions during pPCI in STEMI patients with multivessel disease was based on angiographic severity of the lesion (diameter stenosis of 50 % or more). The invasive PCI procedure (stenting) in NSTEMI patients was based on angiographic severity of the lesion (diameter stenosis of 50 % or more), exclusively for up to 72 hours from the onset of myocardial ischemia symptoms. The invasive PCI procedure in UAP patients was performed electively based on angiographic severity of the lesion (diameter stenosis of 50 % or more).

Outcomes

MACE, as a primary end point, was defined as all deaths, nonfatal myocardial infarction, nonfatal cerebrovascular insult (CVI) and repeated PCI or need for CABG that were assessed within one-year observation. After a one-year follow-up, patients were divided into two groups: patients with MACE and patients without MACE.

Variables – risk factors

Following risk factors were recorded for all the patients: age, gender, categories of ACS, categories of DM (type 1 or type 2), therapy of DM (with insulin or without insulin), glycosylated hemoglobin (HbA1c) in %, HbA1c ≥ 8% (unregulated DM), total cholesterol (mmol/L), total cholesterol ≥ 4.5 mmol/L (high level in diabetes), triglycerides (mmol/L), triglycerides ≥ 2.3 mmol/L (high or very high level), the SYNTAX score, the SYNTAX score > 21 and modified ACEF score.

Estimated glomerular filtration rate (eGFR) was calculated using Cockroft-Gault formula. Renal insufficiency was defined as eGFR < 60 mL/min. Left ventricular ejection fraction (LVEF) was assessed by echocardiographic examination in the first 24 h after pPCI, using Simpson's biplane method. Severe LVEF was defined as LVEF < 40%.

Modified ACEF score was calculated using the formula: years/EF +1 point for every 10 mL/min reduction in CrCl lower than 60 mL/min to 1.73 m^2 (to maximum 6 points). Therefore, CrCl between 50–59 mL/min to 1.73 m^2, 49–49 mL/min to 1.73 m^2, 30–39 mL/min to 1.73 m^2 will get 1, 2 or 3 points. The criterion value of modified ACEF for MACE detection was evaluated after completion of the study and was also used as a predictor variable of the above outcomes.

SYNTAX score was calculated in the manner described by Brkovic et al. [7]. All angiographic variables pertinent to calculation were computed by two interventional cardiologists who were blind to procedural data and clinical outcome. In case of disagreement, the opinion of the third observer was obtained, and the final decision was made by consensus.

Table 2

Frequency distribution by category variables with the significance of the difference between MACE patients' groups

Variables	Patients with MACE		*p* (χ^2)
	No (n = 81) f1 (%)	Yes (n = 55) f2 (%)	
Gender			
male	54 (66.7)	31 (56.4)	0.279
female	27 (33.3)	24 (43.6)	(0.223)
Acute coronary syndrome			
STEMI	30 (37.0)	14 (25.5)	0.152
NSTEMI	7 (8.6)	10 (18.2)	(3.768)
UAP	44 (54.3)	31 (56.4)	
DM – type			
1	2(2.5)	2(3.6)	1.000
2	79(97.5)	53(96.4)	
DM – therapy			
with insulin	23 (28.4)	21 (38.2)	0.265
without insulin	58 (71.6)	34 (61.8)	(0.231)
HbA1c ≥ 8 %			
no	46 (56.8)	13 (23.6)	0.000
yes	35 (43.2)	42 (76.4)	(14.659)
Triglycerides ≥ 2.3 mmol/L			
no	56 (69.1)	35 (63.6)	0.579
yes	25 (30.9)	20 (36.4)	(0.447)
Triglycerides ≥ 2.3 mmol/L in patients with insulin therapy			
no	78 (96.3)	47 (85.5)	0.050
yes	3 (3.7)	8 (14.5)	
Triglycerides ≥ 2.3 mmol/L in patients without insulin therapy			
no	59 (72.8)	43 (78.2)	0.480
yes	22 (27.2)	12 (21.8)	(0.499)
Total cholesterol ≥ 4.5 mmol/L			
no	11 (13.6)	11 (20.0)	0.349
yes	70 (86.4)	32 (80.0)	(0.996)
Left ventricular ejection fraction < 40%			
no	70 (86.4)	23 (58.2)	0.000
yes	11 (13.6)	11 (41.8)	(13.931)
Modified ACEF score > 1.38			
no	61 (75.3)	18 (32.7)	0.000
yes	20 (24.7)	37 (67.3)	(24.396)
SYNTAX score > 21			
no	61 (75.3)	14 (25.5)	0.000
yes	20 (24.7)	41 (74.5)	(32.915)
pPCI			
no	0 (0.0)	6 (42.9)	0.000
yes	30 (100)	8 (57.1)	
Stenting in NSTEMI patients			
no	0 (0.0)	6 (60.0)	0.035
yes	7 (100)	4 (40.0)	
Stenting in UAP patients			
no	2 (4.5)	23 (74.2)	0.000
yes	42 (95.5)	8 (25.8)	

DM – diabetes mellitus; HbA1c – glycosylated hemoglobin; STEMI – acute myocardial infarction with ST elevation; NSTEMI – acute myocardial infarction without ST elevation; UAP – unstable angina pectoris; pPCI – primary percutaneous coronary intervention; ACEF – age, creatinine, ejection fraction.

Statistical methods

Continuous numerical data sets were described by the mean and standard deviation. The attributive or ordinal variables were described by the frequency of outcomes and percentages. Univariable analysis was performed using Pearson χ^2 test or Fisher's exact test for categorical variables and Student *t*-test for continuous variables.

Binary logistic regression method with stepwise variable selection was used for multivariate analysis of MACE risk factors. All variables that had a $p < 0.1$ on univariable analyses were considered for inclusion in the final model.

The evaluation of the validity of the logistic regression model implied an assessment of its goodness-of-fit measure and its accuracy. Goodness-of-fit model was made by estimating the Nagelkerke R Square. The accuracy of the logistic regression model was assessed using discrimination and adequacy. Discrimination measures were conducted to prove how adequately a model can distinguish patients with MACE from patients without MACE. The analysis of the adequacy of logistic models and the estimation of the retention of variables or their interactions were made using the Hosmer-Lemeshow method. Discrimination validity of the predicted probabilities, obtained by logistic regression model and/or the newly constructed Major Adverse Cardiac Events Risk Index (MACERI), in distinguish MACE positive vs. MACE negative patients was estimated by Receiver Operating Characteristic (ROC) procedure. "Cut point" value, sensitivity, specificity, positive predictive value and negative predictive value were obtained by applying maximum Youden index. The testing of the significance of differences between the areas under the curve (AUC) or c statistic of the newly constructed MACERI in relation to the AUCs of other scales was performed by the DeLong method.

The accepted level of significance was $p \leq 0.05$. The statistical program IBM SPSS Statistics 20 (NY) was used for the data processing and MedCalc 12.5.0 (Belgium).

Development of MACERI

Once the model was developed using the regression equation, it was used to develop MACERI. We used the method described in the Framingham study [25] for conversion of the parameter of estimated regression model into an index. The number of points assigned to each variable equaled its regression coefficient divided by 0.5, followed by rounding to the nearest whole number. The points for each risk factor were then summed to obtain the total number of points (score) for a patient. Formula for back-transforming from logistic regression estimated score to probability was as follows:

$$\text{Estimated probability percentage} = 100 \times \frac{e^{logit}}{1 + e^{logit}}$$

where natural logarithm is presented with "e".

Results

The study included 136 consecutive patients with ACS and DM who were all subjected to PCI, of which 55 patients developed at least one MACE in a one-year follow-up period; 28/55 (50.9%) of the patients had two or more MACE. In the period of one year, 25/55 (45.45%) of patients underwent CABG treatment, while a repeated PCI was performed in 13/55 (23.64%) of the patients. 8/55 (14.55%) of the patients died, while 9/55 (16.36%) of the patients developed CVI. All patients involved in the study had multivessel CAD. Detailed descriptions of the study population characteristics are shown in Tables 1 and 2. Using the ROC procedure, we estimate a "cut point" for the modified ACEF > 1.38 (Table 3).

Compared to the non-MACE patients' group, patients with MACE were older and had higher values of the SYNTAX score, higher values for the modified ACEF scores, as well as all higher values for variables used to calculate the modified ACEF (Table 1). Patients with MACE also had higher average values of HbA1c (%) than the non-MACE patients. There was no differences between the groups of patients in the total cholesterol level (mmol/L) and level of triglycerides (mmol/L) (Table 1).

Compared to the non-MACE patients, the patients with MACE had more frequent unregulated diabetes (HbA1c > 8%) and cardiac insufficiency (LVEF = 40%), more frequent the SYNTAX score > 21 and the modified ACEF score > 1.38 and also less frequent pPCI and less frequent stenting in UAP patients and NSTEMI patients (Table 2). By

Table 3
Receiver operating characteristic (ROC) curve analysis of SYNTAX score, modified ACEF score and MACERI in the detection of major adverse cardiac events

Variables	AUC (95% CI)	SE for AUC	*p* (Z)	Cut point (95% CI)	SN (%) (95% CI)	SP (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Modified ACEF score	0.744 (0.662–0.815)	0.045	0.000 (5.472)	> 1.38 (1.21–1.38)	62.7 (53.3–79.3)	75.31 (64.5–84.2)	64.9 (51.1–77.1)	77.2 (66.4–85.9)
SYNTAX score	0.798 (0.720–0.862)	0.041	0.000 (7.318)	> 21 (18.8–24.5)	74.55 (61.0–85.3)	75.31 (64.5–84.2)	67.2 (54.0–78.7)	81.3 (70.7–89.4)
MACERI	0.879 (0.812–0.929)	0.029	0.000 (12.070)	> 7 (5–7)	78.18 (65.0–88.2)	86.42 (77.0–93.0)	79.6 (66.5–89.4)	85.4 (75.8–92.2)

AUC – area under the curve; SE – standard error; CI – confidence interval; Z – normal distribution zed value; SN – sensitivity; SP – specificity; PPV – positive predictive value; NPV – negative predictive value; MACERI – major adverse cardiac events risk index; ACEF – age, creatinine, ejection fraction.

Table 4

Parameters for major adverse cardiac events risk on multivariable analysis with assigned points

Variables in the Equation	B	SE	Wald	df	*p*	Odds ratio	95% CI for Odds ratio		Appropriate points
							lower	upper	
Modified ACEF score > 1.38 (no) – reference									0
Modified ACEF score > 1.38 (yes)	1.952	0.521	14.054	1	0.000	7.044	2.538	19.546	4
Acute coronary syndrome			5.823	2	0.054				
STEMI – reference									0
NSTEMI	1.247	0.801	2.423	1	0.120	3.479	0.724	16.717	0
UAP	1.387	0.592	5.484	1	0.019	4.004	1.254	12.785	3
SYNTAX score > 21 (no) – reference									0
SYNTAX score > 21 (yes)	2.197	0.498	19.499	1	0.000	9.002	3.394	23.874	4
HbA1c ≥ 8% (no) – reference									0
HbA1c ≥ 8% (yes)	1.032	0.484	4.541	1	0.033	2.806	1.086	7.250	2
Insulin therapy by triglycerides ≥ 2.3 mmol/L (no) – reference									0
Insulin therapy by triglycerides ≥ 2.3 mmol/L (yes)	2.523	0.897	7.911	1	0.005	12.471	2.149	72.369	5
Constant	-4.164	0.790	27.761	1	0.000	0.016			

HbA1c – glycosylated hemoglobin; STEMI – acute myocardial infarction with ST elevation; NSTEMI – acute myocardial infarction without ST elevation; UAP – unstable angina pectoris; SE – standard error; CI – confidence interval.

using univariable testing, in all remaining variables no significant difference was found in the distribution of outcome rates between patients' groups.

By applying multivariable regression analysis, we identified four independent risk factors and one interaction between two risk factors for MACE and after which we made the allocation of appropriate points to form the MACERI (Table 4): 1) the modified ACEF score > 1.38; + 4 point; 2) UAP; + 2 points; 3) the SYNTAX score > 21; + 3 points; 4) HbA1c ≥ 8%; + 2 points, and 5) the interaction between insulin terapy and triglycerides ≥ 2.3 mmol/L; + 5 points. The above model showed moderate level of goodness-of-fit measure (Nagelkerke R Square = 0.537) and very good discriminating characteristics (Table 4) and adequacy (Hosmer-Lemeshow test χ^2 = 8.271; p = 0.219). The MACERI > 7 was the criteria for detection of very high MACE risk, that corresponded with estimated probability (or individual patient risk) for the MACERI > 0.44 (Tables 3 and 5). Based on 95% confidence interval for MACERI "cut point" in MACE detection (Table 3), we formed three levels of risk for the patient, as a low risk, increased risk and very high risk (Table 5). The average MACERI in the non-MACE patients' group was 4.65 ± 3.14, while in the group of patients with MACE was 9.62 ± 2.85.

By DeLong method, we found the difference in the AUC for the MACERI *versus* the AUC for the SYNTAX score (Difference between areas = 0.0814; 95% confidence interval from 0.00748 to 0.155; z = 2.158; p = 0.031). Also,

Table 5

Major adverse cardiac events risk index with risk levels categorization and estimated risk percentage *per* total points for a patient

Total points for a patient	Estimated risk percentage for a patient	Risk categorization by levels	Patients (n = 136) n (%)
0	1.53		
1	3.80		
2	4.18	Low risk	40 (29.4)
3	5.86		
4	9.87		
5	14.87		
6	23.50	Increased risk	42 (30.9)
7	30.47		
8	49.63		
9	61.11		
10	73.44		
11	79.30		
12	87.48		
13	92.47	Very high risk	54 (39.7)
14	95.15		
15	99.17		
16	99.98		
17	99.99		
18	99.99		

we found the difference in the AUC for the MACERI *versus* the AUC for the modified ACEF score (Difference between areas = 0.135; 95% confidence interval from 0.0522 to 0.218; z = 3.195; $p = 0.0014$). However, there was no difference in the AUC for the SYNTAX score compared to the AUC for the modified ACEF score (Difference between areas = 0.0536; 95% confidence interval from -0.0580 to 0.165; z = 0.942; $p = 0.346$).

Discussion

In our study, we first performed an assessment of the association of metabolic, anatomic –angiographic and clinical risk factors for the development of one-year MACE in patients with ACS and DM, who underwent PCI. We found that there was a combined impact of these risk factors in the study population and formed MACERI composed of the following significant risk factors: HbA1c > 8%, triglicerydes > 2.3 mmol/L in patients with insulin therapy, UAP diagnosis, the SYNTAX score > 21 and the modified ACEF score > 1.38.

In terms of HbA1c, our results support the recommendations of the American Diabetes Association (ADA) that in patients with DM and advanced microvascular (including ACS) and macrovascular complications, the target HbA1c values should be less restrictive (HbA1c < 8%) [26]. As the same ADA criteria were set for patients with frequent development of hypoglycaemia, ADA wants to prevent further compromise of multivessel CAD that would result from potential development of hypoglycaemia in patients with poor glucoregulation. Bearing in mind the "cut point" for the MACERI estimated in this study, we note that strict adherence to ADA recommendations potentially can avoid one-year MACE in patients with ACS and DM, even in cases where they additionally have the SYNTAX score > 22 or the modified ACEF score > 1.38.

In our study, a very high risk of one-year MACE in patients with insulin therapy and triglycerides ≥ 2.3 mmol/L was assessed. Since this risk is completely independent of the risk arising from HbA1c ≥ 8 %, this is the result of suboptimal insulin patients' therapy and their lifestyle. Therefore, we consider that correction of patient's lifestyle would be unsuccessful, and that they require intensive treatment with statins and fibrates at the same time in accordance with the recommendations of the European Association for Cardiovascular Prevention and Rehabilitation [27]. However, if these patients also have HbA1c ≥ 8%, there is a need to optimize their insulin therapy with the permanent measurement of glycemic profiles. This last point is highlighted taking into account "cut point" and scoring system for the MACERI that indicates that patients with insulin therapy and triglycerides ≥ 2.3 mmol/L associated with HbA1c ≥ 8% have a very high risk of a one-year MACE development, even when they have the SYNTAX score ≤ 22 and the modified ACEF score ≤ 1.38.

We evaluated that patients with UAP compared to the reference group of patients with STEMI had an increased risk for one-year MACE. The reason for this is seen in the reduced rate of invasive PCI in these patients compared to the patients with STEMI (28.8% vs. 57%, respectively). Therefore, in the future, it is important to consider more precise criteria for the implementation of invasive PCI in patients with UAP and DM, as our results suggest that more frequent stent placement in these patients could be beneficial in terms of reducing the risk of one-year MACE after PCI. It is also suggested that this risk in patients with UAP in relation to the reference category of patients with STEMI can be indirectly associated with a rarely implemented invasive PCI. Namely, all patients who underwent the invasive PCI certainly had a permanently monitored dual aggregation therapy in the observed one-year follow-up.

In this study, it was shown that there was no difference in AUC for the SYNTAX score versus AUC for the modified ACEF in the detection of risk of a one-year MACE. A similar result was demonstrated in Pivato et al. [24], who analyzed a mixed population with/without DM and ACS, but also a one-month follow-up after PCI. In any case, we showed that the MACERI had significantly better ROC characteristics, both in relation to the modified ACEF score and in relation to the SYNTAX score.

In the review of Yadav et al. [28], they emphasized the necessity to precisely determine the "cut point" for the SYNTAX score for MACE detection in patients with DM and ACS with longer follow-up period after PCI. We estimated that the "cut point" for the SYNTAX score in the one-year MACE was 22 (18.8 to 24.5). In the reports of other authors, the values for the SYNTAX score defined by the mentioned interval were similar, both in the prediction of MACE [17, 29], and in the assessment of associated risk factors in severe CAD forms [30, 31].

Study limitation

The limitations of this study are single center design, relatively small sample size, and non-inclusion in the evaluation of variables such as stent number, stent types, left main disease, culprit-only PCI and others. The performance of our studies, including the formation of attitudes of a variety of methods during PCI, and the definition of repeat revascularization as adverse events, corresponds to the time when said attitudes are still applicable and have been the subject of controversy [32]. However, such attitudes have now been overcome. As we did a cross-sectional study of risk factors for the development of MACE in the population of patients treated with PCI at that time, then we could not ignore this fact by simply avoiding repeat revascularization within MACE. We emphasize that our study was not an intervention study, hence there was no design, sample size, power and methodological basis for a clear assessment between the various procedures in the course of the PCI.

The major limitation of the SYNTAX score is that it estimates only anatomical complexity and distribution of coronary artery disease. The SYNTAX II score combines

clinical and anatomical risk estimation, but needs still to be validated in prospective cohorts.

Conclusion

Major adverse cardiac events risk index is highly adequate in predicting the most important one-year adverse events in patients with diabetes mellitus and acute coronary syndrome who underwent percutaneous coronary intervention. This index combines the risks that arise from metabolic, angiographic and clinical variables, and as such is more accurate in the prediction of mentioned events compared to the scales of which it is composed.

Conflict of interest

Authors declare that they have no conflict of interest.

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Public trust and media influence on anxiety and depression levels among skilled workers during the COVID-19 outbreak in Serbia

Uticaj poverenja javnosti i medija na nivoe anksioznosti i depresije među stručnim radnicima tokom COVID-19 epidemije u Srbiji

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Abstract

Background/Aim. Along with the great impact of 2019 coronavirus disease (COVID-19) on physical health, social functioning, and economy, this public health emergency has significant impact on mental health of people as well. The aim of this study was to assess the impact of outbreak-related information and public trust in the health system and preventive measures during the COVID-19 outbreak in Serbia in 2020 on levels of anxiety and depression in education, army and healthcare professionals. **Methods.** An anonymous questionnaire was disseminated to skilled professionals working in fields of education, army, and healthcare. The questionnaire included the Beck Anxiety Inventory, Zung Self-Rating Depression Scale, as well as the section assessing the perceived disturbance by the outbreak-related information and the trust of participants in healthcare system and preventive measures proposed by the crisis team. **Results.** Out of 110 subjects enrolled in this study (mean age 35.25 ± 9.23 years), 59.1% were women. Among healthcare workers, the frequency of perceiving outbreak-related information available in public media as disturbing, as well as the average level of anxiety, were higher compared to the group of army professionals ($p < 0.05$). Women also perceived outbreak-related information available in public media as disturbing in a higher percentage compared to men ($p < 0.01$), and had higher levels of anxiety ($p = 0.01$) and depression ($p < 0.05$). The lack of public trust was associated with higher levels of depression, and the perception of outbreak-related information as disturbing with higher levels of both anxiety and depression. **Conclusion.** Significant perception of outbreak-related information as disturbing among healthcare workers, as well as the lack of trust in healthcare system and preventive measures proposed by the crisis team are important factors influencing the mental state. This finding has the guiding purpose for competent institutions to make efforts to increase public trust, as one of the important preventive measures, in order to preserve and improve the mental well-being of the population in outbreak conditions.

Key words:
anxiety; communications media; covid-19; depression; medicine, preventive; mental health; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Pored velikog uticaja bolesti izazvane koronavirusom krajem 2019. godine (COVID-19) na fizičko zdravlje, socijalno funkcionisanje i ekonomiju, ovaj javnozdravstveni problem ima značajan uticaj i na mentalno zdravlje ljudi. Cilj rada bio je da se proceni uticaj informacija povezanih sa epidemiološkom situacijom i poverenja u zdravstveni sistem i preventivne mere tokom COVID-19 epidemije u Srbiji u 2020. godini na nivoe anksioznosti i depresije kod radnika obrazovne, vojne i zdravstvene struke. **Metode.** U studiji je korišćen anonimni upitnik koji je prosleđen stručnim radnicima iz obrazovnog, vojnog i

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sistema zdravstvene zaštite. U upitniku su korišćeni Bekova skala anksioznosti, Cungova skala samoprocene depresivnosti, kao i deo koji su kreirali autori i koji je ispitivao doživljaj uznemirenosti ispitanika informacijama koje se odnose na stanje epidemije i poverenje ispitanika u zdravstveni sistem i mere prevencije propisane od strane kriznog štaba. **Rezultati.** Od ukupno 110 ispitanika, uključenih u ovu studiju, prosečne starosti 35,25 ± 9,23 godina, 59,1% su činile žene. Kod zdravstvenih radnika su učestalost doživljavanja informacija u vezi sa stanjem epidemije, dostupnih putem medija, okarakterisanih kao uznemirujuće, kao i prosečan nivo anksioznosti, bili viši u odnosu na zaposlene u vojsci ($p < 0,05$). Žene su takođe doživljavale kao uznemirujuće javno dostupne informacije u vezi sa stanjem epidemije u višem procentu u poređenju sa muškarcima ($p < 0,01$), a imale su i prosečno više nivoe anksioznosti ($p = 0,01$) i depresije ($p < 0,05$). Nedostatak poverenja javnosti je bio povezan sa višim nivoima depresije, a doživljavanje informacija u vezi sa stanjem epidemije kao uznemirujućih, sa nivoima i anksioznosti i depresije. **Zaključak**. Izraženo doživljavanje informacija u vezi sa stanjem epidemije kao uznemirujućih među zdravstvenim radnicima, kao i značajno odsustvo poverenja u zdravstveni sistem i u preventivne mere predložene od strane kriznog štaba, predstavljaju značajne činioce sa uticajem na mentalno stanje. Ovakav nalaz ima zadatak da dodatno usmeri odgovorne institucije ka naporima da povećaju poverenje javnosti, kao jednu od važnijih preventivnih mera, u cilju očuvanja i unapređenja mentalnog blagostanja stanovništva u uslovima epidemije.

Ključne reči:
anksioznost; mediji, komunikacijski; covid-19; depresija; medicina, preventivna; mentalno zdravlje; ankete i upitnici.

Introduction

Within weeks of its initial outbreak in China, 2019 coronavirus disease (COVID-19) has been declared as public health emergency of international concern [1–5].

Following the first officially confirmed case of COVID-19 in Serbia on March 6, 2020, the response efforts by the Serbian Government have been swift, and seven days later the COVID-19 crisis response team has been formed [6]. The crisis team began making recommendations based on the scientific knowledge of the situation to limit social contacts, encourage wise use of medical supplies including masks and other personal protective equipment, and to assure the public about the reliability of the food and consumable goods supplies.

Furthermore, only nine days after the first confirmed case in Serbia, in an unprecedented move to retard the spread of the virus, an emergency state has been declared in the whole country. It included many restrictions, along with several travel restrictions and lockdowns over the whole territory of Serbia. Four days after the state of emergency had been declared, the Ministry of Construction, Transport and Infrastructure of the Serbian Government ordered that all commercial international flights from and to the Belgrade Nikola Tesla Airport to be suspended on the same day [7].

The longest lockdown was imposed over the citizens older than 65 years of age during the whole day for almost two months. Another most constant type of lockdown was imposed during the every night over all citizens, with an exception of those with nonreplaceable working duties. Also, several constant lockdowns were imposed, some of them lasting for even four days, and mostly during the weekends and public or religious holidays. Beside citizens older than 65 years of age, many other people stayed at home and socially isolated themselves in order to prevent being infected. There have also been accounts of shortages of masks, gloves, antiseptics, other health equipment, and some basic foodstuff.

Based on our understanding, the vast majority of the research related to this pandemic focuses included identification the epidemiology and clinical characteristics of infected patients [8, 9], the genomic characterization of the virus [10], and challenges for global health governance [11].

However, the ongoing COVID-19 epidemic is inducing fear, and a timely understanding of mental health status is of paramount importance [12]. Previous research has revealed a wide range of impacts on psychosocial wellbeing of people during many previous outbreaks of infections in the world. Fear of falling sick or dying, feelings of helplessness, and stigma are the most common effects people are likely to experience on an individual level during the outbreaks [13]. With the closure of schools and business, negative emotions experienced by individuals are additionally compounded [14].

The COVID-19 pandemic also caused panic and mental health problems for the public [15, 16], like it was experienced previously with the Middle-East respiratory syndrome (MERS) coronavirus [17, 18].

Additionally, myths and misinformation about this epidemic, travel bans and executive orders to quarantine travelers affected the psychological health of the majority of the mankind even more and this has severe influence on people's health and quality of life [15, 19, 20].

Although several recent research articles examined the influence of COVID-19 outbreak on mental health in general population [21, 22], there are no published papers describing the impact of individual aspects of this emergency situation on some determinants of mental state – anxiety and depression, especially among members of professions specific by their significant importance for security and proper functioning of countries.

Therefore, the aim of this study was to evaluate the influence of information related to the epidemiological situation and public trust in healthcare system and preventive measures proposed by the epidemic crisis team during the COVID-19 outbreak in Serbia in 2020 on anxiety and

depression as determinants of mental state in skilled education, healthcare and army professionals.

This study was a cross-sectional online survey designed to assess the prevalence and the degree of anxiety and depression, the absence of public trust in proposed preventive measures, as well as to indirectly establish the connection between the presence of controllable and uncontrollable factors, since several previous studies focused their goals on this specific topic [23–26].

This study was the first survey describing the psychological impact of COVID-19 outbreak on mental state, conducted in the population of skilled workers in Serbia during the COVID-19 outbreak.

Methods

Setting and participants

We adopted a cross-sectional survey design to assess experiencing disturbance by the epidemic-related factors, public's trust in health system and levels of anxiety and depression during the epidemic of COVID-19 by using an anonymous online questionnaire. A snowball sampling strategy [27], focused on recruiting skilled workers in healthcare, military and education systems, was utilized. The online survey was at first disseminated to the healthcare workers, university professors and military workers in Serbia known to the authors and they were encouraged to recruit other subjects from their environment and to forward the survey to them. Healthcare workers specialized or working in the field of mental healthcare were excluded from the study.

Procedure

As the Serbian Government recommended the public to minimize face-to-face interaction and isolate themselves at home, potential respondents were electronically invited by the authors or the existing study respondents. They completed the questionnaire in Serbian language through an online survey platform ("Google Docs", Google LLC, Mountain View, California, United States). Data collection took place in several cities in Serbia during seven days (July 13–19, 2020).

Prior to accessing the survey, participants read an informed consent statement describing that participation was voluntary and anonymous. An information sheet stating the goal and the procedure of the study was also presented to participants at the beginning of the survey. After that, the participants entered the survey and answered the questions. Besides the participation was based on voluntarity, no incentive reward was given. Anonymity was emphasized and no identifiable information was collected.

Survey development

The structured questionnaire consisted of questions that covered five areas: 1) demographic data; 2) the Beck Anxiety Inventory (BAI) [28]; 3) the Zung [29] Self-Rating Depression Scale (SDS); 4) information related to the experience of disturbance caused by the epidemic-related information available in public media or its absence; 5) information related to the participant's trust in the Serbian healthcare system and preventive measures proposed by the crisis team.

Demographic data including gender, age and occupation were collected. A question anout occupation was constructed as multiple choice question. Participants had to choose one of four answers: education worker, emplyed in military service, doctor of medicine, and nurse/medical technician.

Age was included to determine differences in perceived disturbances caused by the epidemic-related information, public trust in the healthcare system and the preventive measures proposed by the crisis team, as well as in BAI and SDS scores. Specific reason for including age in the analysis was based on the data of some reports which essentially called out different age groups for ignoring public health recommendations [30, 31].

Assessment of anxiety and depression

Anxiety was assessed using the BAI [28]. Participants rated each of the 21 items on a 4-point Likert scale [32] from 0 (not at all) to 3 (most of the time). Total BAI score ranges from 0–63. Scores up to the value of 21 were classified as low anxiety, scores of 22–35 as moderate anxiety, and scores of 36 and above as potentially concerning levels of anxiety.

Depression was assessed using the Zung's [29] SDS. Participants rated each of the 20 items on a 4-point Likert scale from 1 (rarely) to 4 (most of the time) or *vice versa*, depending on the positivity and negativity of the questions. Total SDS score ranges from 20–80. Scores within the range of 20–49 were classified as normal state, scores of 50–59 as mild depression, scores of 60–69 as moderate depression, and scores of 70 and above as severe depression.

Questions concerning disturbing epidemic-related experience and public trust

The part of the questionnaire evaluating perceived disturbance in relation to the epidemic-related information, as well as the trust in the healthcare system of Serbia and the preventive measures proposed by the Serbian crisis team, was consisted of the following six dichotomous questions created by the authors – During the outbreak, have you experienced disturbance by:

1) Media reports regarding the outbreak?

2) The information from other sources you have learned by your own initiative?

3) The lack of the information regarding the COVID-19 outbreak and the disease itself?

4) The possibility of virus transmission from other people despite personal preventive measures you applied?

5) During the outbreak, have you expressed trust in the healthcare system?

6) The preventive measures proposed by the crisis team?

Data analysis

Methods of descriptive statistics were used in analysis of sociodemographic characteristics of respondents, anxiety and depression scores, questions regarding the disturbance caused by the epidemic-related factors, and public trust questions. The scores of BAI and SDS were expressed as mean and standard deviation (SD).

The χ^2 test was used to determine the difference between categorical variables, while *t*-test and analysis of variance were used to determine differences in BAI and SDS scores among groups based on gender, age, occupation, the presence of disturbing factors related to the epidemic, and the presence/absence of public trust.

All tests were two-tailed, with a significance level of $p < 0.05$. Statistical analysis was performed using the software platform SPSS Statistics version 26.0 (IBM SPSS Statistics, New York, United States).

Results

Sociodemographic characteristics of examined subjects are shown in Table 1.

Table 1

Sociodemographic characteristics of respondents, perceived disturbance, public trust, and levels of anxiety and depression

Variables	All respondents (n = 110)	Occupation				
		Education (n = 36)	Army (n = 41)	Healthcare		
				all (n = 33)	medical doctors (n = 21)	nurses/technicians (n = 12)
Gender						
male	45 (40.9)	11 (30.6)	29 (70.7)	5 (15.2)	4 (19.0)	1 (8.3)
female	65 (59.1)	25 (69.4)	12 (29.3)	28 (84.8)	17 (81.0)	11 (91.7)
Age (years)						
21–30	42 (38.2)	9 (25.0)	18 (43.9)	15 (45.5)	8 (38.1)	7 (58.4)
31–40	40 (36.3)	13 (36.1)	14 (34.2)	13 (39.3)	10 (47.6)	3 (25.0)
41–50	20 (18.2)	9 (25.0)	8 (19.5)	3 (9.1)	2 (9.5)	1 (8.3)
51–60	7 (6.4)	4 (11.1)	1 (2.4)	2 (6.1)	1 (4.8)	1 (8.3)
61–70	1 (0.9)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Perceived disturbance caused by the epidemic–related factors						
information in public media	70 (63.6)	25 (69.4)	20 (48.8)	25 (75.8)	16 (76.2)	9 (75.0)
information available from other sources	55 (50.0)	19 (52.8)	17 (41.5)	19 (57.6)	13 (61.9)	6 (50.0)
absence of information	57 (51.8)	15 (41.7)	22 (53.7)	20 (60.6)	12 (57.1)	8 (66.7)
possibility of transmission despite personal preventive measures	75 (68.2)	24 (66.7)	27 (65.9)	24 (72.7)	17 (81.0)	7 (58.3)
Trust						
in the healthcare system	72 (65.5)	24 (66.7)	29 (70.7)	19 (57.6)	13 (61.9)	6 (50.0)
in the preventive measures proposed by the crisis team	62 (56.4)	21 (58.3)	22 (53.7)	19 (57.6)	13 (61.9)	6 (50.0)
Anxiety level						
low	99 (90.0)	34 (94.4)	37 (90.2)	28 (84.9)	20 (95.2)	8 (66.7)
moderate	9 (8.2)	1 (2.8)	4 (9.8)	4 (12.1)	1 (4.8)	3 (25.0)
potentially concerning	2 (1.8)	1 (2.8)	0 (0.0)	1 (3.0)	0 (0.0)	1 (8.3)
Depression level						
normal	97 (88.2)	31 (86.1)	38 (92.7)	28 (84.9)	17 (81.0)	11 (91.7)
mild	12 (10.9)	5 (13.9)	3 (7.3)	4 (12.1)	4 (19.0)	0 (0.0)
moderate	1 (0.9)	0 (0.0)	0 (0.0)	1 (3.0)	0 (0.0)	1 (8.3)

***Note*: Results are given as number (percentage) of respondents.**
BAI – Back Anxiety Inventory; SDS – Self-Rating Depresion Scale.

A total of 110 respondents completed the survey, 59.1% of whom were women and 40.9% men. Participants were on average 35.25 years of age, ranging from 22 to 62. Most participants were up to 40 years of age (n = 82, 74.6%) and the most represented category was 21–30 years-old (n = 42, 38.2%). For all participants, it took up to five minutes to complete the survey. Occupation groups were similar according to the number of subjects within each group. Distribution of the participants by occupation is presented on Figure 1.

Respondents' depression level, measured using the SDS [29], revealed a sample mean score of 39.29 (SD = 9.17), while their anxiety level, measured using the BAI [28], had a value of sample mean score of 10.08 (SD = 9.29). Analyzing the depression scale scores, 97 (88.2%) of the respondents were considered to have a normal score (score: 20–49), 12 (10.9%) had mild depression (score: 50–59), and one participant (0.9%) had moderate depression (score: 60–69). There were no participants having score of severe depression. According to the results of the anxiety scale, 99 (90.0%) of the participants were considered to have a low-level anxiety (score: 0–21), nine (8.2%) were considered to suffer from moderate-level anxiety (score: 22–35), and two (1.8%) from potentially serious levels of anxiety (score of 36 or above).

The majority of respondents (70.0%) declared that they were disturbed by the information distributed on public media. Half of all participants felt disturbance by the information they learned on their own initiative. A slight majority (51.8%) of participants expressed their opinion that there was the lack of available information in public and felt disturbance accordingly. Additionally, 68.2% of the subjects were disturbed by the fact that they can be infected despite their protective measures and as a consequence of irresponsible behavior of other people.

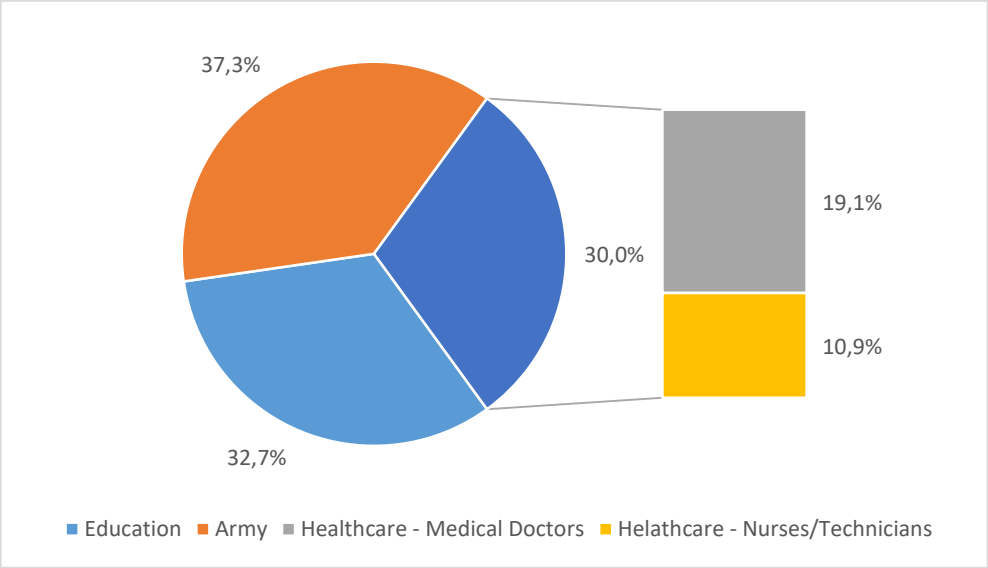

Fig. 1 – Distribution of participants by occupation (n = 110).

Regression analysis revealed the connection between the perceived disturbance and BAI/SDS scores. Respondents who experienced disturbance by the information available on public media had significantly higher BAI score (B = 0.40, 95% confidence interval (95% CI): 3.58 to 11.75), while those who did not have trust in health system had higher SDS score (B = -0.27, 95% CI: -9.49 to -0.74). Linear regression did not show any significant impact of age on perceived disturbance.

Considering specific occupations, the highest number of participants perceiving disturbance by the fact that they can be infected despite their protective measures was noticed among medical doctors (81.0%). On the other side, 75.0% of nurses and medical technicians were disturbed by the information stated in public media, followed by disturbance caused by the absence of reliable information (66.7%) (Table 1).

Additionally, significant difference in perceived disturbance by the information stated on public media was also observed among different occupation groups ($p < 0.05$) (Table 2) Participants from the education and healthcare systems as occupation reported disturbances caused by the information stated on public media in larger extent (69.4% and 75.8%, respectively) than army employees (48.8%) (Table 1).

Table 2

The influence of demographic characteristics on perceived disturbances caused by the epidemic-related factors, public trust and anxiety/depression levels

Variables	Gender		Age group		Occupation	
	χ^2	p	χ^2	p-value	χ^2	p
Perceived disturbances caused by the epidemic–related factors						
information in public media	7.157	0.007**	2.098	0.718	6.530	0.038*
information available from other sources	3.046	0.081	6.852	0.144	2.064	0.356
absence of information	2.809	0.094	4.284	0.369	2.562	0.278
possibility of transmission despite preventive measures	0.018	0.895	2.099	0.718	0.455	0.797
Trust						
in the healthcare system	0.397	0.529	4.523	0.340	1.434	0.488
in the preventive measures proposed by the crisis team	0.284	0.594	3.644	0.456	0.198	0.906
Anxiety level	1.692	0.429	2.951	0.937	3.406	0.492
Depression level	1.051	0.591	2.885	0.941	3.309	0.508

***$p < 0.05$; **$p < 0.01$**

Perceived disturbance by the information stated on public media was also present in significantly larger percent (73.8%) among women comparing to male participants ($p < 0.01$). Out of all skilled workers as participants, 72 (65.5%) expressed trust in the healthcare system of Serbia, and 62 (56.4%) in the preventive measures proposed by the expert crisis team. Among healthcare workers specifically, exactly half of the participating nurses and technicians declared to have the trust in both aspects equally. Linear regression did not show any significant impact of age on public trust.

The significant difference with regard to the mean BAI score was observed among different occupation groups ($p < 0.05$), between army and healthcare workers (Table 3). Within the group of healthcare workers, statistically significant difference was detected between medical doctors and nurses/technicians only in the BAI score ($F = 4.843$; $p = 0.035$).

Table 3

Group comparisons of BAI and SDS scores according to demographic characteristics, perceived disturbance and public trust

Variables	n (%)	BAI score			SDS score		
		mean (SD)	*t* test/ F test	*p*	mean (SD)	*t* test/ F test	*p*
Gender							
male	45 (40.9)	7.47 (7.928)	*t* = -2.516	0.013**	36.91 (8.764)	*t* = -2.310	0.023*
female	65 (59.1)	11.89 (9.781)			40.94 (9.144)		
Age (years)							
21–30	42 (38.2)	10.55 (9.688)			40.21 (9.291)		
31–40	40 (36.3)	8.78 (8.034)			38.58 (8.802)		
41–50	20 (18.2)	11.50 (11.464)	F = 0.443	0.777	39.15 (10.464)	F = 0.202	0.937
51–60	7 (6.4)	11.43 (8.059)			38.71 (9.810)		
61–70	1 (0.9)	5.00			36.00		
Occupation							
education	36 (32.7)	11.06 (9.168)			40.19 (9.089)		
army	41 (37.3)	6.95 (7.899)	F = 4.299	0.016*	37.10 (9.044)	1.977	0.144
healthcare	33 (30.0)	12.91 (10.110)			41.03 (9.139)		
Perceived disturbance caused by the epidemic–related factors	70 (63.6)	12.51 (9.908)	*t* = -4.353	0.000**	40.69 (8.878)	*t* = -2.145	0.034*
information in public media	55 (50.0)	11.98 (10.212)	*t* = -2.182	0.031*	39.31 (9.135)	*t* = -0.021	0.984
information available from other sources	57 (51.8)	10.63 (8.053)	*t* = -0.642	0.522	41.07 (8.610)	*t* = -2.145	0.034*
absence of information possibility of transmission despite preventive measures	75 (68.2)	10.57 (8.509)	*t* = -0.811	0.419	39.37 (9.382)	*t* = -0.137	0.891
Trust							
in the healthcare system	72 (65.5)	9.13 (9.099)	*t* = 1.495	0.138	37.38 (8.565)	*t* = 3.137	0.002**
in the preventive measures proposed by the crisis team	62 (56.4)	9.02 (8.794)	*t* = 1.373	0.173	37.73 (8.769)	*t* = 2.065	0.041*

BAI – Back Anxiety Inventory; SDS – Self-Rating Depresion Scale; SD – standard deviation.
***p* < 0.05; ***p* < 0.01.**

The mean BAI score was also significantly higher in female participants ($p = 0.01$), and subjects perceiving disturbance by the information stated in public media ($p < 0.01$) and the information available from other sources ($p < 0.05$).

SDS scores were significantly different between groups based on gender, perceiving disturbance by the epidemic-related information stated in public media and the absence of available epidemic-related information, and trust in healthcare system and in the preventive measures proposed by the crisis team. In general, females, participants perceiving disturbance by the epidemic-related information stated in public media and the absence of available epidemic-related information, as well as those with the lack of trust in the healthcare system and in the preventive measures proposed by the crisis team, had higher SDS score (Table 3).

Regression analysis did not show any significant impact of age on both BAI/SDS scores and levels of anxiety/depression. On the other side, it was shown that male gender was significantly associated with lower BAI (B = 0.24, 95% CI: 0.94 to 7.91) and SDS scores (B = 0.22, 95% CI: 0.57 to 7.48).

Discussion

Emergency state is not a foreign term for Serbia. This country faced similar situations many times during the last three decades, but none of them was related to the epidemic. However, this kind of adaptability is somewhat specific, due to the fact that lockdown was not seen in Serbia since bombing in 1999 and considering the fact that, even back then, the lockdowns were imposed only when necessary, for a short periods of time, and were not obligatory. However, such an intensive outbreak, requiring an emergency state, has not been seen since the smallpox epidemic in 1972.

According to the Oxford Government Response Tracker, Serbia was among the countries with the highest calculated stringency level from mid-March to mid-May 2020 [33]. During that period of time, Serbia imposed all five main physical distancing policies: school closures, workplace closures, restrictions on mass gathering, public transport closure, and lockdown [34]. All these measures, combined with increasing number of tested citizens effectively lead to the slowing down of infection rate, and reducing mortality, and numbers proved continuously more positive environment, which was later confirmed by the numerous opinions of several countries in the world, showing that the preparedness level of Serbia was at the very high level [35, 36].

However, all these measures impacted greatly on mental health of overall Serbian population, as has been the case all over the world. Significant connection between several characteristics and anxiety/depression (BAI/SDS) scores observed in this study is strongly suggestive towards the influence of factors concerning healthcare system in Serbia and the information provided by the officials and on public media. Although the majority of participants were 40 years old or younger, a quarter of them were older than 40

years of age and represented a sample large enough to contribute to the overall initial conclusion regarding the impact of the Serbian healthcare system impression on the skilled workers in Serbia and their perceiving of epidemic-related information as disturbing. The fact that the majority of respondents perceived disturbance by the information distributed on public media, and that half of them reported epidemic-related information from other sources as disturbing certainly originates to large extent from the presence of sudden changes in overall epidemic state in Serbia. The beginning of the second wave of the epidemic state in Serbia at the end of June 2020 certainly introduced severe confusion among people, even among skilled workers. One of the study conducted by the regional marketing consulting and research company "Valicon" revealed that two thirds of participants from general population did not have trust in the information provided by the crisis team and the state officials [37]. More than a half of participants in the present study expressed trust in the preventive measures proposed by the expert crisis team, while almost two thirds of them expressed trust in the Serbian healthcare system. Specific finding was that half of all participating nurses and medical technicians in this study expressed trust in both aspects, and other half did not.

Population habits in Serbia are one of the most important factors for the finding that almost 70% of all participants in our study reported disturbance by the fact that they can be infected despite their protective measures and as a consequence of irresponsible behavior of other people. Additionally, perceived disturbance by the epidemic-related information available on public media was connected with the higher levels of anxiety, while the absence of trust in the healthcare system was related to the higher levels of depression. Although average anxiety and depression levels were not very high, the influence of the epidemic-related information available on public media and loss of trust in the healthcare system had significant influence on overall mental state of the population examined in this study. Loss of trust reported in this study could not be connected with the lockdown-period, due to the fact that it was conducted out of such a restraining circumstances, although some studies reported that people in the pandemic/lockdown group had higher trust in science, politicians, and police [38].

However, the finding that was not expected at the beginning of this study to appear was the perceived disturbance by the epidemic-related information available on public media which was reported by three quarters of nurses and medical technicians, as well as the disturbance caused by the absence of available information perceived by the two thirds of them.

Findings of this study suggest that female participants are prone to be significantly more disturbed by the information stated on public media than males, which certainly lead to the higher BAI scores in women. However, employees in the Serbian Army perceived significantly less disturbance by the information stated on public media and smaller BAI scores comparing to other skilled workers investigated in this study. This finding might be the result of the fact that army employees were in direct contact with (potentially) COVID-19 positive individuals in significantly smaller extent than healthcare workers, and certainly due to their protected position by the country.

It is well known that anxiety level has influence on many aspects of behavior. During the SARS coronavirus epidemic in 2003, Leung et al. [39] found that moderate levels of anxiety were associated with higher uptake of preventive measures by respondents. In contrast, findings published by Wang et al. [21] showed the opposite trend, specifically for COVID-19 outbreak in China. This specific aspect was not tested in this study, but it certainly could be very good point of investigation in some future studies.

Regarding depression level, our data suggest that factors associated with higher SDS score were female gender, perceiving disturbance by the epidemic-related information available in public media and the absence of available epidemic-related information, as well as the lack of trust in the healthcare system and in the preventive measures proposed by the crisis team. The finding of this study that females suffered from higher level of depression on average corresponds to previous extensive epidemiological studies suggesting that women are at higher risk of depression [40], and particularly with the findings suggesting a greater psychological impact of the outbreak, as well as higher levels of stress, anxiety, and depression in females [21].

Although this study was the first one connecting epidemic-related factors to the mental state, as well as the mental state itself and public trust in Serbia, it was not conducted on general population. This study did not include any impact-on-event scale in order to strengthen the connection between potential factors and current mental state. This survey did not measure stress levels among participants as well. All these limitations are very correctable, especially due to the fact that this study should represent the basis for future research, with even more participants of different age groups, in longer follow-up, and in successive periods of time, so that complete image of mental health timeline during the epidemic environment in Serbia could be created. Also, the same potential causes and their effects could be assessed using random, systematic, stratified, or some other types of sampling in order to eliminate all the biases following every single sampling technique.

Additionally, this study was conducted on three types of skilled workers, chosen due to the considerable change in their everyday work caused by the epidemic state. Psychosocial impact of disturbing factors related to epidemic, public trust in healthcare system and officially proposed preventive measures, as well as other factors which could be considered as controllable and uncontrollable can and should be also tested on skilled workers of many other professions, especially the ones with significant impact of proper functioning of the country during the emergency state like this.

One additional and very important topic is fear during the COVID-19 outbreak [41]. Fear of being infected and fear from dying are among the most commonly seen

types of fear which rose in intensity during the public health emergency like this one. One another specific type is fear of using cardiopulmonary resuscitation (CPR) as a response to cardiac arrest. Unfortunately, there are reported cases of fatal outcomes in people with cardiac arrest outside of hospital setting where bystanders did not initiate CPR due to the fear that they could be infected [42]. While it is understandable to be concerned by the novel coronavirus, laypeople should be encouraged to start at least continuous chest-compression-only CPR or to use automated external defibrillator [43, 44]. Therefore this kind of fear should certainly be investigated as well in some future research.

Also, the fact that this study was performed during the second wave of the outbreak in Serbia unfortunately leave in the shadow valuable information regarding the perceiving epidemic-related disturbing factors, public trust, and anxiety/depression levels during the first wave. Although this fact will remain permanent, it can be concluded with significant level of certainty that the results provided by this study are very significant, especially as the basis for future investigations.

Conclusion

Higher anxiety and depression levels in women, perceived disturbance by the epidemic-related information, especially among some healthcare workers, as well as the significant absence of trust in healthcare system and proposed preventive measures in public could be the most important conclusive findings of this study, and certainly the factor that should represent guideline for responsible institutions in Serbia and all over the world to increase efforts of individuals and especially governments in improving public trust up to the highest possible level, due to its high impact on perceiving any epidemic-related information on more positive way. Our findings can also be used to formulate additional and more effective psychological interventions in order to improve mental health and psychological resilience during the COVID-19 epidemic. Finally, findings in this study may also assist government agencies and healthcare professionals in safeguarding the psychological wellbeing of the community in the face of COVID-19 outbreak in Serbia, surrounding region, and many different parts of the world, especially in other middle-income countries.

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GENERAL REVIEW

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Testicular cancer stem cell hypothesis – diagnostic and therapeutic implications

Hipoteza stem ćelija testikularnog karcinoma – dijagnostičke i terapijske implikacije

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Ključne reči:
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Introduction

Testicular cancer is an uncommon malignancy in men and is curable in most instances. There is marked geographical variation with age-standardized rate (ASR) *per* 100,000 ranging from 4.6 in developed countries to 0.8 in developing countries [1]. The incidence of testicular cancer is highest in Western and Northern Europe, Australia, and North America, while the lowest incidence is in Asia and Africa [2]. Despite its low overall incidence, it is the most common cancer in young men, in the third or fourth decade of life [3]. The incidence of testicular cancer has been increasing over the past four decades, while mortality rate has been decreasing in most European countries [4–6]. Over 71,000 new testicular cancer cases and 9,500 deaths are estimated to occur worldwide in 2018 [7].

According to the World Health Organisation, testicular tumours can be pathologically classified into seven categories: germ cell tumours derived from germ cell neoplasia *in situ*, germ cell tumours unrelated to germ cell neoplasia *in situ*, sex-cord stromal tumours, tumour containing both germ cell and sex-cord stromal elements, miscellaneous tumours of the testis, haematolymphoid tumours, and tumours of collecting duct and rete testis [8]. More than 90% of all testicular cancers are germ-cell tumours almost equally divided in seminomas and nonseminomas [9]. Seminomas are consisted of classic seminoma, spermatocytic seminoma and intratubular germ cell neoplasia [10]. Seminomas are composed of transformed germ cells that closely resemble the primordial germ cells (PGCs) or gonocytes that are blocked in differentiation and can not undergo normal spermatogenesis. Non-seminomas include embryonal carcinomas, yolk sac tumours, teratomas and choriocarcinomas [10]. Nonseminomas can contain different histological elements due to pluripotency of the PGCs or gonocytes, normally only apparent after fertilization (Figure 1) [11]. Seminomas are most frequent in the fourth decade of life, while nonseminomas peak in the third decade of life [11].

Testicular cancer has some unique biological features different from other solid tissue cancers. First, it has unusual histology with components that mimic any tissue type of the body. It can be explained by the fact that testicular cancer originates from germ cells that use two different types of cell division (mitosis and meiosis) [12]. Second, germ cells preserve embryonic stem cell features and pluripotency for a long period during development [12].

Aetiology and pathogenesis

Testicular cancer has a largely unexplained aetiology. There are a number of risk factors for testicular cancer associated with prenatal or perinatal exposures, including low birth weight, low maternal parity, cryptorchidism, infertility, family history, and white race. A systematic review and meta-analysis of perinatal factors in relation to

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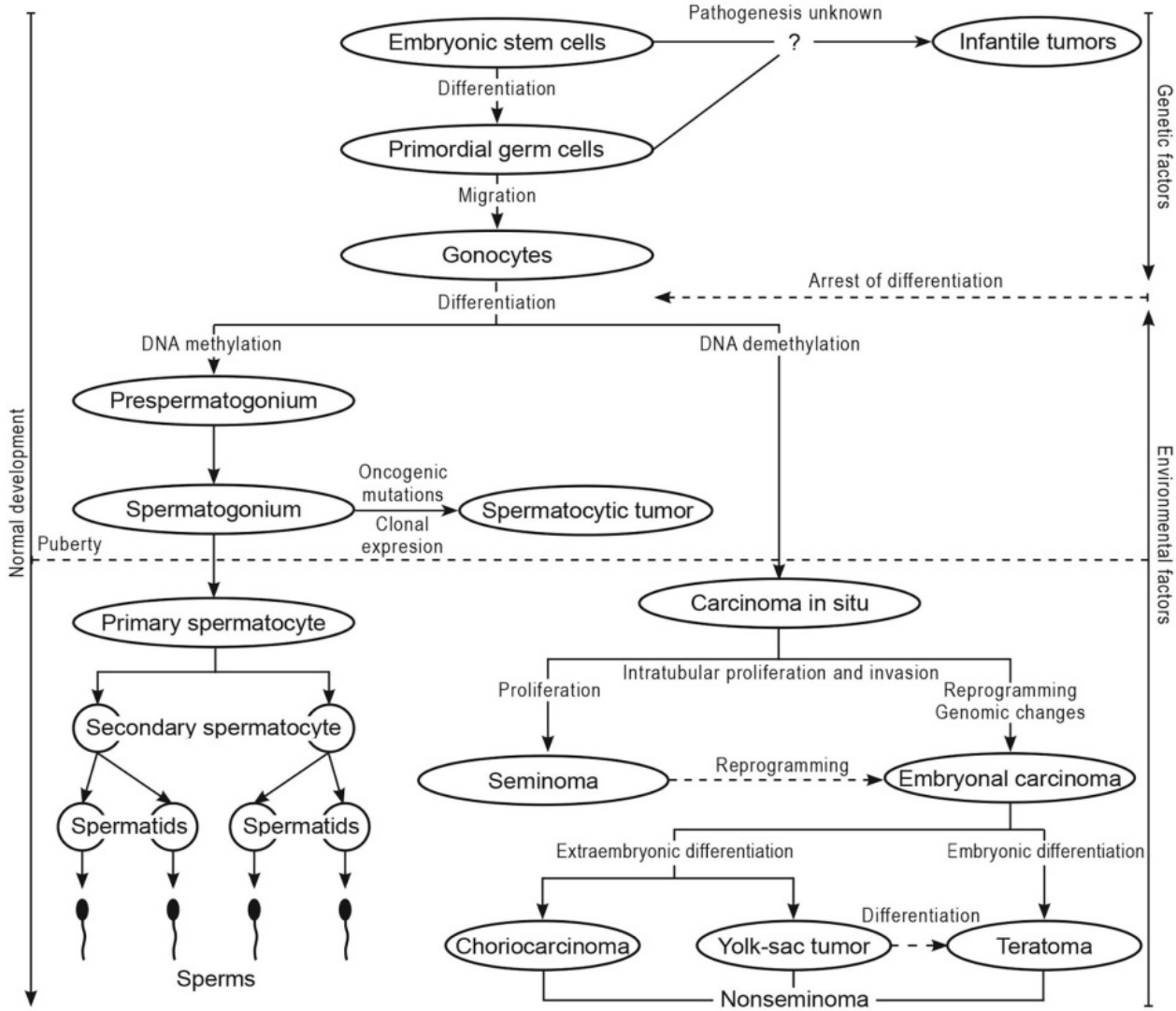

Fig. 1 – Normal germ cell development and model of histogenesis of the testicular germ-cell tumors.

the risk of testicular cancer found evidence that cryptorchidism, inguinal hernia, twinning, birth weight, and gestational age are associated with increased risk of testicular cancer [13]. It is speculated that oxidative stress can also be a cause [14, 15]. However, while spermatozoa are much more susceptible, spermatogonia are highly tolerant to oxidative stress [16, 17].

During testicular carcinogenesis preinvasive cells gave rise to overt tumour. Testicular cancer is derived from cells in the germ cell lineage that are blocked in maturation. The pre-invasive stage of testicular germ-cell tumour (TGCT) of adolescents and young adults is carcinoma *in situ* (CIS) or intratubular germ cell neoplasia unclassified, which is thought to arise from malignant transformation of a PGCs or gonocytes [18]. Skakkebaek [19] described atypical spermatogonia in testicular biopsies from two patients who later developed overt testicular tumours. There is evidence that approximately 50% of patients diagnosed with CIS of the testis develop invasive testicular cancer within 5 years of diagnosis [20]. The incidence of testicular tumours that develop from CIS has increased during last decades. There are three other very rare types of TGCT which develop without CIS stage: spermatocytic seminoma, teratomas, and yolk sac tumours that have remained at steady low incidence level [21].

Normal germ cell development

PGCs in mammals are committed to secure transmission of genetic information to the next generation by production of mature oocytes in females and spermatozoa in males. They arise at the base of allantois at an early stage of embryogenesis in week 5 to 6. Thereafter, they migrate toward both genital ridges to the places where the gonads will develop. When they rich gonadal ridges, they are called gonocytes. Embryonic germ cells are characterized by several markers including placental alkaline phosphatase (PLAP), octamer-binding transription factor (OCT4), and NANOG. Differentiation of the gonocytes into either oogonia or prespermatogonia depends on chromosomal constitution and microenvironment. PGCs are in fact the

totipotent stem cell population of the body [22]. PGCs are considered the stem cells of oogenesis in female and spermatogenesis in male.

Stem cells

Stem cells are undifferentiated cells that possess ability to self-replicate and to differentiate into mature cells of the organ in which it resides [23, 24]. There are three groups of stem cells, ie., embryonic, germinal and somatic stem cells. Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst and are the precursors of all cells in our body. Germinal stem cells in the adult produce eggs and sperm and are responsible for reproduction. Somatic stem cells are responsible for normal tissue renewal [25].

The different types of stem cells proliferate differently. ESCs divide symmetrically, whereby each daughter cell retains the properties of the parental cells resulting in a logarithmic expansion of cells [25]. Germinal and somatic stem cells divide asymmetrically, whereby one daughter cell remains a stem cell (self-renewal), and undergoes expansion and further differentiation into mature cells, whereas the other daughter cell becomes progenitor cell that undergoes expansion and further differentiation into mature cells [25].

Stem cells can be obtained from the embryo or from extraembryonic tissues such as the umbilical cord blood obtained at birth, the amniotic fluid, and the placenta. Stem cells can be found in adult mammals in bone marrow, blood, skin, and testis. ESCs are thought to be pluripotent with ability to differentiate into a variety of cell types. ESCs exposed to certain conditions differentiate into cell types of all three germ layers (endoderm, ectoderm and mesoderm) as well as into germ line cells. Adult stem cells (ASCs) are more conservative in their proliferation and differentiation. Adult stem cells are limited to the tissue in which they reside [26]. Stem cells have much longer life span and therefore have greater opportunity to gather genetic mutations [27]. It has been postulated that stem cells can be transformed into cancer if signalling pathways that regulate their renewal become disrupted.

Cancer stem cells

There are two models of carcinogenesis, “stochastic” and “stem cell”. In “stochastic” model of carcinogenesis any cell may be target of random mutation. Growth of tumours is assigned to the serial acquirement of genetic events that resulted in the turning on the genes promoting proliferation, turning of genes inhibiting proliferation, and surrounding of genes responsible for apoptosis [28]. The “stem cell” model suggests that cancers originate in tissue stem cells through deregulation of self-renewal processes. In this model of carcinogenesis, the key event is disruption of genes responsible for self-renewal [28]. The idea that precursors for a growing list of cancers are cancer stem cells (CSCs) is almost 150 years old [29]. It has been speculated that tumours are initiated and maintained by a population of cancer cells with stem cell properties known as CSCs. CSCs were first described in acute myeloid leukemia [30]. Surface markers were used to distinguish the stem cells from the rest of cells with limited proliferative potential [30]. The CSCs hypothesis became essential for understanding the carcinogenesis and developing strategies for cancer prevention. CSCs are characterized as a minor cell population able to sustain themselves by self-renewal and to generate committed progenitors that gradually form solid tumour. CSCs model is based on the idea that the vast majority of tumour cells have moderate proliferative potential compared to a small cell population – the CSCs, which are able to self-renew and proliferate in order to maintain tumour cell mass [31]. Consequently, cancer is a disease of deregulated self-renewal of normal stem cells [32]. In this model, tumour restitution and even metastases may happen due to residual chemotherapy resistant cells [32]. Normal stem cells and cancer cells share several important properties like the ability of self-renewal, activation of antiapoptotic pathways, and the ability to migrate and metastasize [29]. Stem cells are subjected to the multiple mutations required for carcinogenesis during their life cycle. Adolescent women exposed to atomic bomb radiation in Hiroshima and Nagasaki developed breast cancer 2 to 3 decades after exposure. It is thought that in that period, the mammary gland has the highest number of stem cells [33].

There is accumulating evidence that CSCs exist in a spectrum of tumours. It has been hypothesised that testicular CIS cells resemble CSCs in our body [21]. CIS cell is pluripotent and has capability to develop into variety of germ cell tumours. There is evidence that CIS is precursor of TGCT. Frequent finding of CIS in testicular parenchyma surrounding invasive cancer, as well as the development of invasive TGCT in patients in whom CIS has previously been diagnosed support hypothesis. It has been hypothesized that arrest in development and differentiation of the early germ cell lineage is the main pathogenetic mechanism that leads to neoplastic transformation into CIS [21]. These cells are localised in the seminiferous tubules located between the basal membrane and the Sertoli cell layer. CIS can be found in all risk populations for TGCT more frequently, as well as in the surrounding tissue of TGCT than in testes of healthy men. CIS cells are thought to be remnants of undifferentiated foetal cells. Soon after their discovery, similarity to gonocytes was noted. Imunohistochemical studies found that CIS cells and foetal germ cells contain large amounts of glycogen and PLAP, the most commonly used marker for detection of CIS cells.

The precise mechanism underlying the transformation of the gonocyte to CIS and further into overt testicular tumour is mainly unknown. It is thought that testicular cancer is initiated during foetal development. It was hypothesized that CIS cells originate from PGCs or gonocytes that have failed to differentiate into spermatogonia as a consequence of endocrinological imbalances [12]. PGCs are thought to be changed either during migration to the embryonic genital ridges or after cells have arrived at the gonads. Little is known about the behaviour of CIS cells after birth. It is likely that they are inactive during infancy, starting to replicate after puberty, possibly as a consequence

of new hormonal conditions progressing to overt tumours. Prevalence of CIS in the general population of young adults is not known. It has been estimated that the prevalence of CIS is the same as lifetime risk of testicular cancer [34].

Diagnostic approach

"Stem cell" model for cancer will likely improve diagnosis and treatment of cancer. If the CSC hypothesis is valid, than we need to discover new tumour markers made by CSCs for early detection of cancer. Testicular cancer is potentially fatal and its treatment has severe side-effects. Therefore, efforts should be made to establish diagnosis at early, preinvasive CIS stage. At present, there is no imaging technique or serological method for the diagnosis of CIS which is asymptomatic. It can be diagnosed only by a surgical biopsy [18, 35]. CIS cells can be routinely identified in biopsies by morphologic and immunohistologic characteristics. Appropriate fixatives (Stieve's or Bouins solution) should be used to diagnose CIS cells in paraffin sections. Formalin fixation should be avoided [36]. Morphologically, CIS cells are located in a single row at the basement membrane of seminiferous tubules. Cells are larger in diameter containing larger nucleus than that of normal spermatogonia. There is a large amount of glycogen in the cytoplasm of CIS cells. Therefore, their cytoplasm appears optically empty on histological sections since glycogen is washed out during fixation.

Additional immumohistochemical staining by using several antibodies like PLAP [37] or OCT3/4 [38] is an advanced option for detecting CIS cells. The monoclonal antibodies M2A and 43/F are highly sensitive for detecting CIS immunohistochemically [39]. The immunohistochemical tumour markers TRA 1-60 [40] and neuron specific enolase [41] are expressed in the majority of CIS cells. The proto-oncogene c-kit protein product Kit is over expressed by CIS cells [42]. The possible foetal origin of CIS is supported by immunohistochemical studies of proteins present in CIS, that are also present in PGCs and gonocytes [21]. Tra-1-60 and M2A which are present in great quantity in CIS but undetected in the adult testis were also detected in normal foetal and infantile germ cells [43]. Kit is strongly expressed in early foetal germ cells up to 12 weeks of gestation [36]. Differentiation of gonocytes into infantile spermatogonia begins around 20 weeks of gestation and in some cases end prenatally, but quite often continues in the early postnatal period until 6 to 9 months of age, when the markers that are shared by PGC, gonocytes, and CIS (PLAP, OCT4, NANOG) are finally down-regulated [21, 43]. Persistent expression of these markers later in childhood is not normal [44].

Therapeutic implications

The CSCs hypothesis opens new possibilities for cancer prevention and treatment, as well as predicting the cases at high risk for metastasis [45]. Traditionally, drugs used for cancer treatment are directed against proliferating cells. Testicular cancers are among the most sensitive solid cancers to chemotherapy [46, 47]. However, it is likely that agents selectively killing CSCs are overlooked [46, 48]. Consequently, tumour growth is reinitiated and relapse is plausible. If transformed stem cells are targets of intervention, then treatments that can reduce stem cell number might reduce cancer risk. It can be achieved through induction of apoptosis or differentiation of stem cells. In acute myeloid leukemia and breast cancer, tumorigenic cells are minor part of the tumour bulk. Furthermore, new cancer model has significant impact on our ability to identify individuals at risk for metastasis. It has been hypothesized that only when CSCs disseminate self-renew metastases will occur. Further studies should develop diagnostic tools that will allow us to predict in which cases metastatic disease will develop. It will help clinicians to identify patients that will benefit from chemotherapy and spare patients from unnecessary treatment [49].

Conclusion

CIS is considered precursor of TGCT, and an excellent example of CSCs. Understanding the biology and cellular chemistry of CIS is important for developing new strategies for prevention and treatment of TGCT. Efforts should be made to obtain diagnosis of TGCT at the CIS stage, as early intervention is warranted before an invasive tumour develops. Further research is needed to obtain a method of noninvasive CIS detection. It would make possible to offer to patients timely and optimal treatment.

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Conflict of Interest

The authors have no conflict of interest to declare.

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CASE REPORTS

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Physical therapy improves motion in a patient with inclusion body myositis – A case report

Fizikalna terapija poboljšava kretanje kod bolesnika sa miozitisom praćenim inkluzionim telima

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Abstract

Introduction. Inclusion body myositis (IBM) is a rare form of inflammatory myopathy with a slowly progressive course. It is manifested by early weakness and atrophy of skeletal muscles, especially forearm muscles and the quadriceps. At the very beginning of the disease, clinical symptoms are not pronounced, therefore it is difficult to diagnose**. Case report.** A forty-eight-year-old female patient visited her doctor due to the weakness of muscles in arms and legs. Five years prior to this, she was treated by a neurologist and a physiatrician on several occasions with different diagnoses for progressive muscle weakness. During the last hospitalization, IBM was diagnosed after the muscle biopsy findings. After the diagnosis, the patient underwent intensive physical therapy in order to preserve the ability to independently perform everyday activities and stability of walk. **Conclusion.** IBM is a rare clinical entity which often takes several years to be diagnosed. Progressive muscle weakness in elderly should point to possible IBM diagnosis, which is only confirmed by muscle biopsy. Physical therapy has a significant role in the treatment as it leads to improvement of functional abilities of the patients in their daily activities, thus reducing the disability degree.

Key words:
myositis, inclusion body; muscle weakness; physical therapy modalities; biopsy; diagnosis; treatment outcome.

Apstrakt

Uvod. Miozitis sa inkluzionim telima (IBM) je redak oblik inflamatorne miopatije koja ima sporo progresivan tok. Manifestuje se ranom slabošću i atrofijom skeletne muskulature, posebno mišića podlaktice i kvadricepsa. Na samom početku bolesti, klinički simptomi nisu izraženi, pa je postavljanje dijagnoze teško. **Prikaz bolesnika.** Bolesnica stara 48 godina javila se lekaru zbog slabosti u mišićima ruku i nogu. Prethodnih pet godina je u više navrata lečena od strane neurologa i fizijatra sa različitim dijagnozama progresivne mišićne slabosti. Tokom poslednje hospitalizacije, IBM je dijagnostikovan nakon biopsije mišića. Nakon uspostavljene dijagnoze, bolesnica je prošla intenzivnu fizikalnu terapiju kako bi se očuvala sposobnost samostalnog obavljanja svakodnevnih aktivnosti i stabilnost hoda. **Zaključak.** IBM predstavlja redak klinički entitet kod koga često prođe i više godina do postavljanja dijagnoze. Progresivana slabost mišića kod starjih osoba treba da ukaže na moguću dijagnozu IBM, koja se jedino potvrđuje biopsijom mišića. Fizikalna terapija ima značajnu ulogu u lečenju jer dovodi do poboljšanja funkcionalnih sposobnosti

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bolesnica u aktivnostima dnevnog života čime se smanjuje stepen invalidnosti.

Ključne reči:
miozitis sa inkluzionim telima; mišićna slabost; fizikalna terapija; biopsija; dijagnoza; lečenje, ishod.

Introduction

Inclusion body myositis (IBM) is a rare clinical entity which is classified in the group of idiopathic inflammatory myopathies [1, 2]. It is a complex disorder of an known etiological cause, but genetic, immunological and environmental factors are considered to have significant influence in the development of the disease. Numerous studies have hypothesized that IBM may be an autoimmune inflammatory muscle disorder. The lack of the adequate response to the administration of conventional therapeutic modalities which are used in the treatment of autoimmune diseases points to the role of other factors in the pathogenesis of this disorder [3, 4]. It is thought that IBM is closely connected to both autoimmune and degenerative processes. Degenerative processes occur in muscle tissue by forming vacuoles, the so called "inclusion bodies", which are the clusters of various unfolded or misfolded proteins. For this reason, it was long believed that IBM was a degenerative disease of muscle tissue [5]. However, recent research has shown that viral infection could be a trigger which induces abnormal response of the immune system and contributes to the development of IBM [6]. Also, some people have genetic predisposition for this disease [7].

IBM is characterised by chronic, progressive weakness and atrophy of skeletal muscles, especially distal parts of the upper and proximal parts of lower extremities, sometimes followed by the weakness of facial muscles and dysphagia [2, 8]. Since muscle weakness is the main clinical symptom, the diagnosing of IBM often lasts for a long time due to rare occurrence of the disease. Pathological-histological confirmation of the existence of inclusion bodies in skeletal muscles is the fundamental diagnostic procedure. The lack of adequate pharmacological therapy for the IBM patients together with the progressive course of the disease reduces the performance of everyday activities and leads to disability and restricted movability [1, 2, 6, 9].

The aim of this paper is to point to possibilities of occurrence of this disease in the patients with long-lasting muscle weakness, as well as emphasize the necessity of muscle biopsy as a fundamental diagnostic indicator. We also demonstrate the significance of physical therapy as the main treatment of the disease.

Case report

A forty-eight-year-old female patient visited a neurologist at the Clinical Centre in Kragujevac for the first time in 2011 for the weakness in arm and leg muscles, which first appeared eight months before. Electromiography (EMG) and nerve conduction studies (NCS) neurography were done and showed moderate to moderately strong demyelination polyneuropathy in the lower and upper extremities, which resembled the findings after acute inflammatory demyelination polyneuropathy (AIDP). The physical therapy, electrical therapy and kinesiotherapy were administered, which considerably improved general status; general motor strength (GMS) was also improved so that the patient was significantly more stable while moving.

In 2012 and 2013, the patient was hospitalized at the Clinics of Neurology and Physical Medicine, Clinical Centre Kragujevac twice each year due to the weakness in upper and lower extremities and impaired general condition with various diagnoses: sensorimotor polyneuropathy, status post AIDP and leukoencephalopathy. Each hospitalization at the Rehabilitation Centre lasted for twenty-one days. The treatment was conducted daily, six days per week. Kinesiotherapy programme included the exercises of diaphragm breathing, the exercises for thorax expanding, active exercises for maintaining general mobility, exercises for coordination of movement and when changing from lying to sitting position. Kinesiotherapy was conducted for the duration of 40–45 minutes. During each hospitalization, the functional abilities of the patient were evaluated by the Health Assessment Questionnaire (HAQ). At the first hospitalization, the value of HAQ score was 1, which pointed to slight decrease in functional abilities during everyday activities (moderate disability). Manual muscle test (MMT) was done at each hospitalization and had the values of –4/5 for the upper extremities (UE), and –3/5 for the lower extremities (LE). EMG and NCS showed significant worsening in relation to the ones, done in 2011. Magnetic resonance imaging (MRI) of the brain and cervical and lumbosacral spine showed old three lacunar infarctions in the right parietal lobe. Anosmia was diagnosed during the examination of an allergologist. In addition, ergometric tests were done, showing low probability for cardiovascular diseases; however, it should be noted that the test was not properly done due to the patient's incorrect walk ("leg shuffling"). The analysis of cardiolipin total antibodies (IgG, IgA, IgM) was negative. All laboratory parameters were within limits of reference values. After each hospitalization, rehabilitation treatment was indicated, combined with physical therapy in ambulatory and spa conditions, which led to better and more reliable motions of the patient.

In 2014 and 2015, the patient was hospitalized several times at the Daily Hospital and the Clinic for Neurology, Clinical Centre Kragujevac, with the following diagnoses: paraparesis flaccida, polyneuropathy specifica and Guilliain-Barre syndrome. The examination of the cerebrospinal fluid revealed normal fluid composition. Laboratory and serological analyses could not specifically indicate the

existence of Guillain-Barré syndrome, but the clinical picture gave rise to suspicion, and high creatine kinase values indicated the possibility of paraparesis flaccida or polyneuropathy specifica. Hypotrophy of the mimic muscles with light reduction of mimic movements was diagnosed together with a slightly dysarthric speech, reliance on help in changing position from sitting to standing, as well as the hypotrophy of all muscles of upper and lower extremities. The administration of 25 g i.v. of immune globulin IgG-7S did not lead to any improvement. The patient was treated with physical therapy (stable galvanization, electrical stimulation, magnetic therapy, diadynamic currents); kinesiotherapy treatment was conducted in order to reinforce global muscle strength (GMS) and improve walking coordination. HAQ questionnaire was 1, MMT for UE was 3/5, while for LE was 2-3/5. The finding of dual energy X-ray absorptiometry (DEX) of the neck and femur, T-score was -1.1, and regions L1–L4 showed osteopenia.

During the last hospitalization at the Clinic of Neurology (Clinical Centre Kragujevac) in September 2016, muscle biopsy showed IBM. A histological finding showed the presence of muscles of altered architecture with clearly visible necrosis and myofagocytes, due to the presence of inflammatory infiltrates. Inflammatory infiltrate was of the dominant T-lymphocyte type (CD4+, CD8+), with numerous histiocytes and CD8+ lymphocytes invading non-necrotic fibers. Both types of fibers were presented with elements of group atrophy, indicating moderate lesion. In many fibers, the presence of rimmed vacuoles was observed (Figure 1), fibers without cyclooxigenase (COX) activity were present, the expression of the major histocompatibility complex – class 1 (MHC-I class) of antigens in the inflammation zone, but also in fibers that were not necrotic, was amplified.

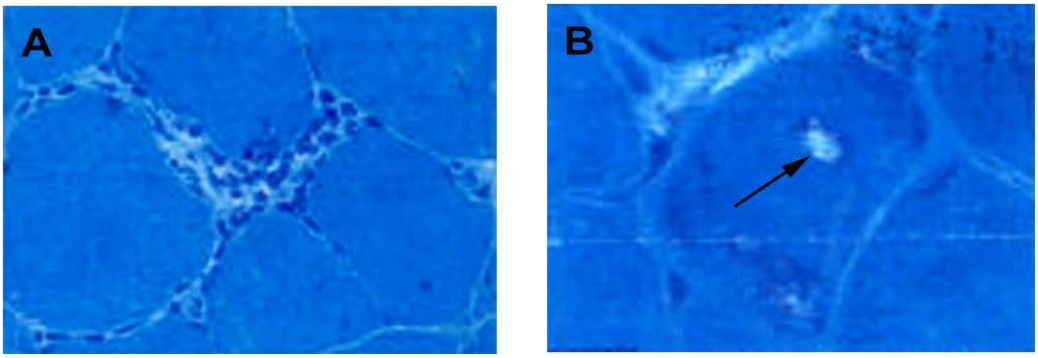

Fig. 1 – Micrography with histological image of the muscle biopsy: A) Myophagocytosis shown on the modified Gomori trichrome staining (10×); B) The presence of a vacuole (arrow), Gomori trichrome staining (40×).

After IBM was diagnosed, the patient was hospitalized at the Centre for Physical Medicine and Rehabilitation, Clinical Centre Kragujevac, where she was administered physical therapy (stable galvanization, transcutaneus electro neural stimulation, diadynamic currents, kinesiotherapy program) and functional testing. MMT was conducted for UE and LE which showed the values of -3/5 and -2/5, respectively. The value of HAQ questionnaire was 2, which pointed to severe disability (Figure 2).

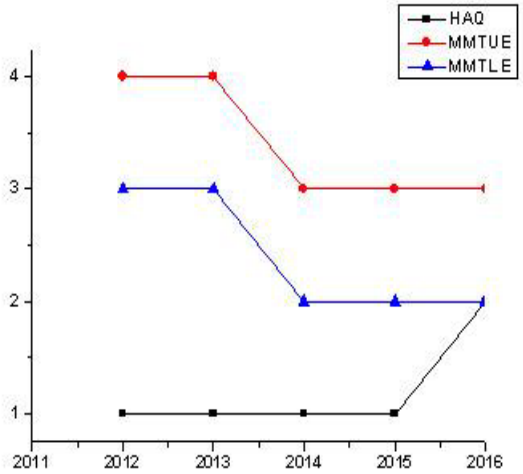

Fig. 2 – The graph shows the effect of physical therapy for a period of 2012–2016 in inclusion body myositis (IBM) measured by Health Assessment Questionnaire (HAQ) and manual muscle testing (MMT).
UE – upper extremities; LE – lower extremities.

During the latter hospitalization, the weakness of body flexors and difficulties with motion in bed were noticed together with difficulties in standing up from the chair; however, the patient walked alone on flat surface with the help of a stick with four bottoming points; kinesiotherapy treatment was conducted in patient's room under the supervision of the therapist with daily exchange of tension and pulsation, since exertion had to be dosed during active exercises. At home, kinesiotherapy included active exercises for general motion and walking on flat surface 2 × 60 minutes, with occasional breaks.

Presentation of the patient's distal parts of upper extremities before and after several cycles of physical therapy is shown in Figure 3.

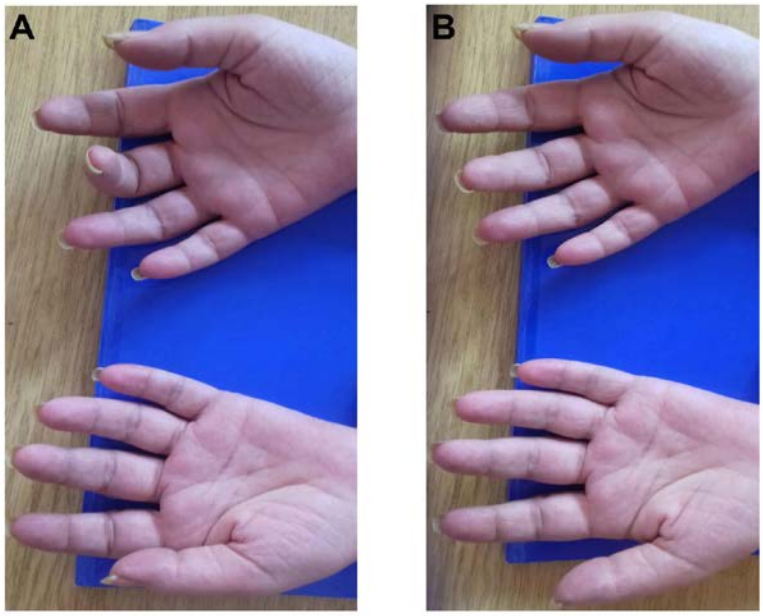

Fig. 3 – Presentation of the patient after the disease was diagnosed: A) Distal parts of upper extremities before physical therapy, and B) after physical therapy.

Discussion

IBM is a rare disease, which more often appears in male in comparison to female population aged over 50, with the ratio 3:1. The frequency of the disease is from 0.001% for those aged less than 50 years to about 0.005% for those aged over 50 [2]. Because of such a low frequency and rare occurrence, several years usually pass before the disease is diagnosed, as was the case with our patient [3].

IBM is characterized by progressive muscle weakness of distal upper extremities and proximal lower extremities for more than a year in the patients aged over 35, and a normal or moderately increased values of creatine phosphokinase (CPK) [2]. In our patient, muscle weakness was a predominant symptom which worsened overtime. In addition to these symptoms, the patient had diagnoses of anosmia and osteopenia, which have been described in other patients with idiopathic myositis [10, 11]. The crucial parameter to confirm the diagnosis of this disease is a muscle biopsy, which was, in our case, performed 5 years after the onset of muscle weakness [1, 4, 7, 9].

Pharmacological therapy has a very small influence on a progressive course of IBM in comparison to other inflammatory myopathies, thus the patients lose the capacity to perform everyday activities and most often end up in a wheelchair 10–15 years after the onset of the disease [2, 4, 9, 12–14]. In our patient, administration of immunoglobulins did not lead to any improvement, while the muscle weakness progressed, so the patients could walk only with the help of aids. The aim of physical therapy was to maintain the function and mobility in all joints, thus providing independent mobility as long as possible. At first, the application of physical therapy in IBM was considered contraindicated due to possible increase of inflammatory process in muscles, but later studies pointed to its significance in muscle strength improvement, endurance and increase of functional abilities of the patients related to movement [1, 15]. After the administration of physical therapy in several cycles, general condition of our patient improved. Mobility was made easier and extremity muscles were slightly strengthened. Her walk was more stable, which improved the quality of her life.

Conclusion

IBM is a rare disease which is often diagnosed after many years. Progressive muscle weakness should point to

possible doubt about IBM, which is only confirmed by muscle biopsy. Physical therapy plays a significant role in the treatment of IBM, since it leads to improvement of the functional capacity of patients in daily activities, thus reducing their disability.

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Mediastinal metastasis of primary extraneural ependymoma: A case report

Primarni ekstraneuralni ependimom – metastaza u medijastinumu

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Abstract

Introduction. The rarity of primary extraneural ependymomas (EnEs), its great variations in morphology and rare occurrence of metastasis, increase chances of misdiagnosis, particularly if they are found in paraovarian localization. **Case report.** The presented patient was diagnosed with malignant mesothelioma 14 years ago, after right salpingo-oophorectomy. In following years patient had multiple and extensive surgical procedures, resulting in different histopathological diagnoses, and after seven years, a diagnosis of EnE was established. Later on, patient was surgically treated in several medical centers across the region, again with different histopathological diagnoses. At present, the tumor metastasized to mediastinum, presenting as a grey to brown, multicystic formation with cysts filled with a clear serous fluid or red-brown hemorrhagic fluid. The inner surface of the cysts had smooth to partly papillary appearance. Tumor cells exhibited several architectural patterns (solid, pseudorosette or rosette formations, papillary and pseudopapilary structures), and immunophenotype specific for EnE [glial fibrillary acidic protein (GFAP), estrogen receptor (ER), and progesterone receptor (PR) positive; calretinin, WT-1, S100, synaptophysin, chromogranin, CK7 and pan-cytokeratin negative]. **Conclusion.** This case demonstrates not only specific diagnostic immunophenotype of extraneural ependymoma, but above all an important principle in tumor pathology. Rare neoplasms may occur in unusual and unexpected primary and metastatic sites. Pathologists need to be familiar with histologic features of a wide range of neoplasms and not just the appearance of neoplasms within their own limited subspecialty area.

Key words:
ependymoma; central nervous system neoplasms; diagnosis; histological techniques; immunohistochemistry; mediastinal neoplasms; neoplasm metastasis.

Apstrakt

Uvod. Osnovni razlog čestih grešaka u dijagnostikovanju ekstraneuralnih ependimoma (EnE), posebno onih lokalizovanih u paraovarijalnoj regiji, jesu njihova niska incidencija, velike varijacije u histomorfološkim odlikama i retka pojava metastaza. **Prikaz bolesnika.** Prikazana je bolesnica kojoj je maligni mezoteliom dijagnostikovan 14 godina ranije, posle desne adneksetomije. Narednih godina, bolesnica je bila podvrgnuta nisu operativnih zahvata u bolnicama u matičnoj državi i u regionu, a na osnovu pregledanog materijala postavljeno je nekoliko različitih patohistoloških dijagnoza. Dijagnoza EnE prvi put je postavljena 7 godina ranije, a posle je postavljeno još nekoliko patohistoloških dijagnoza, bez odgovarajućih analiza u pravcu potvrde ependimoma. Po prijemu u našu ustanovu, bolesnici je konstatovano prisustvo lobulirane promene u medijastinumu, koja je makroskopski bila sivkasto-mrke boje, multicistična. Ciste su bile ispunjene bistro-seroznim do hemoragičnim tečnim sadržajem. Unutrašnjost cisti bila je glatka i delom papilarnog izgleda. Histološki, tumorske ćelije su bile aranžirane u solidne, papilarne i pseudopapilarne formacije, a fokalno su se formirale rozete. Imunofenotip tumorskih ćelija odgovarao je karakteristikama EnE [glijalni kiseli fibrilarni protein (GFAP), estrogenski receptor (ER), progesteronski receptor

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(PR) pozitivne; kalretinin, WT-1, S100, sinaptofizin, Ovaj slučaj ne ilustruje samo specifični dijagnostički imunofenotip ekstraneuralnih ependimoma, već, pre svega, ističe važan princip u tumorskoj patologiji. Retke neoplazme, bilo primarne ili metastatske, mogu se javiti na neuobičajenim i neočekivanim lokalizacijama. Patolozi moraju biti upoznati sa histološkim osobinama širokog hromogranin, CK7 i pan-citokeratin negativne]. **Zaključak**. spektra neoplazmi i to ne samo iz njihovog užeg polja rada.

Ključne reči:
ependimom; nervni sistem, centralni , neoplazme; dijagnoza; histološke tehnike; imunoistohemija; medijastinum, neoplazme; neoplazme, metastaze.

Introduction

Typical ependymomas are rare neuroepithelial tumors originating from the glial ependymal cells of the central nervous system (CNS). A vast majority of ependymomas occurring in adults are localized in the ventricles and spinal cord. Rarely, they occur outside the CNS [extraneural ependymomas (EnEs)], and even than mostly in close relationship with neural axis. The majority of primary EnEs are seen in the sacrococcygeal subcutaneous tissue and paraovarian area, but they have also been reported in the extraovarian pelvic region and more infrequently in other sites [1–4].

We reported the case of a female patient presenting with a mediastinal metastatic deposit of primary EnE.

Case report

A female patient, aged 41, was admitted to the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Vojvodina due to a mediastinal tumor. Since the patient was a foreign national, initially treated in her country and other regional hospitals, the medical documentation was incomplete, and document of histopathological examination was not available.

The patient gave anamnestic data that her initial surgical procedure was right adnexetomy and reduction of tumor mass in pelvic cavity 14 years ago (when she was 27 years old), with histopathological diagnosis of poorly differentiated malignant mesothelioma. Postoperatively, the patient was treated with combined chemoterapy [docetaxel/cisplatin (CDDP) protocol].

At the age of 29, the left ovary cyst was removed, histopathologically diagnosed as a cyst of germinal epithelium, followed by chemotherapy [cisplatin/etoposide/bleomycin (PEB) protocol].

According to patient's anamnesis, at the age of 34, "pseudocyst" from pelvic cavity was removed, and histopathologically diagnosed as well differentiated ependymoma.

At the age of 36, tumorectomy of the left ovary mass and appendectomy were performed, with pathology-confirmed ependymoma metastasizing to the appendix. In the same year, due to multiple metastases, the patient underwent extensive surgery: left adnexectomy, rectosigmoid resection by the Hartmann's procedure, resection of terminal ileum and cecum, right hepatectomy, cholecystectomy, retroperitoneal lympadenectomy, omentectomy, partial peritonectomy, resection of the right hemidiaphragm and tumorectomy for right pleural mass. Chemotherapy (etoposid) was applied postoperatively.

Fine needle aspiration (FNA) of inguinal lymph nodes was performed a year later, at the age of 37, with histopathologically confirmed only reactive changes in lymph nodes. Positron emission tomography/computed tomography (PET/CT) scans in 2014 revealed suspicious lymph nodes near internal mammary artery, which were sampled during video-assisted thoracoscopic surgery (VATS), with pathologically evident tumor metastases. At that time, tumor tissue was composed of medium sized atypical cells, with oval hyperchromatic nuclei, and focally prominent nucleoli. Immunohistochemical analysis showed alpha fetoprotein (AFP) and D2-40 to be positive in all tumor cells; CD30, placental alkaline phosphatase (PLAP) and CK19 were negative. Based on this immunofenotype, diagnosis of metastatic germ cell tumor was set.

Suspicious cystic mediastinal lesion was observed at PET/CT at the age of 40 (2016), and in control PET/CT in next year (2017), somewhat elevated activity/fluoro-deoxyglucose (FDG) accumulation [maximum standard unit value (SUVmax) = 1.7] was observed in the left paracardial area (all imaging analyses were done abroad, and images were not available to us after the patient was sent home). After adequate preoperative procedures, VATS was used to identify and extirpate cystic lesion loosely attached to mediastinum, diaphragm and pericardium, while phrenic nerve was passing over the lesion. Material was sent to histopathological analysis. The post-operative recovery was unremarkable, and the patient was discharged four days later.

Histopathological findings

Material sent for histopathological analysis was grey to brown tissue, lobulated and cystic in its macroscopic appearance, 8×4×2.5 cm large. On cross sections it was multicystic, with cysts filled with clear serous fluid or red-brown hemorrhagic fluid. The inner surface of the cysts had smooth to partly rough and papillary appearance.

Histological slides stained with hematoxylin and eosin (HE) revealed cystic tumor composed of oval, spindle to polygonal tumor cells, with scant clear or light eosinophilic cytoplasm (Figure 1). Nuclei were oval, round and spindle shaped, hyperchromatic, with marked pleomorphism and atypia. Some nuclei had granular chromatin ("salt &

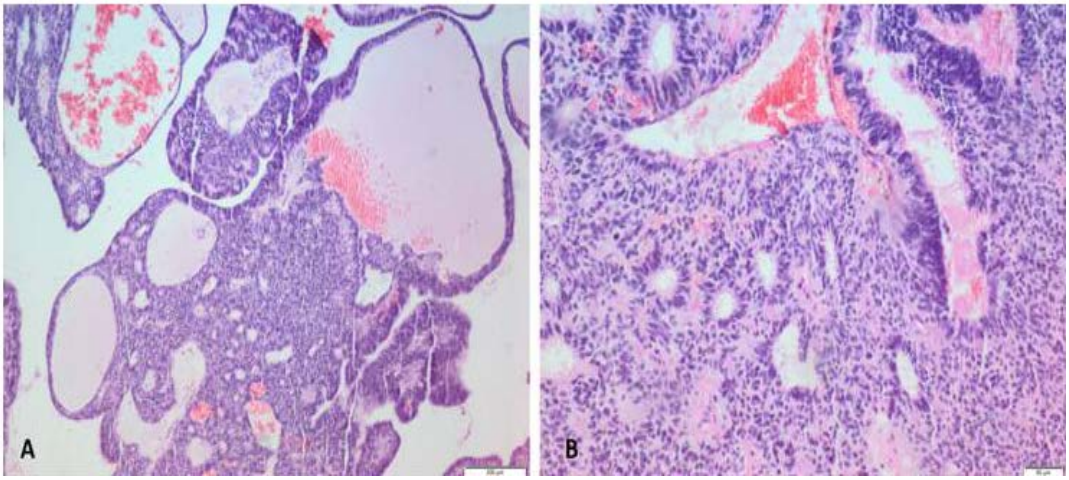

Fig. 1– Photomicrographs of the tumor tissue: A – solid and cystic areas; cysts filled with eosinophilic amorphous material and erythrocytes (HE, 40×); B – oval, spindle and polygonal tumor cells, with scant clear or light eosinophilic cytoplasm, with pseudorosette or rosette formations (HE, 100×).

pepper"). Mitoses were rarely present (2 per 10 high power fields). Cell borders were poorly defined. Tumor cells exhibited different architectural patterns: solid, pseudorosette or rosette formations, papillary and pseudopapilary stuctures. Cystic spaces were filled with eosinophilic amorphous material and erythrocytes. Neither necrosis nor vascular invasion were observed. Based on patient's anamnestic data and histological appearance of the tumor, additional immunohistochemical analysis was indicated. Tumor cells showed strong glial fibrillary acidic protein (GFAP) immunoreactivity, as well as estrogen receptor (ER) and progesterone receptor (PR) immunoreactivity (Figure 2). Less than 5% of cells showed positive Ki-67 nuclear staining (Figure 2). Tumor cells were negative for calretinin, WT-1, S100, synaptophysin, chromogranin, CK7 and pan-cytokeratin staining.

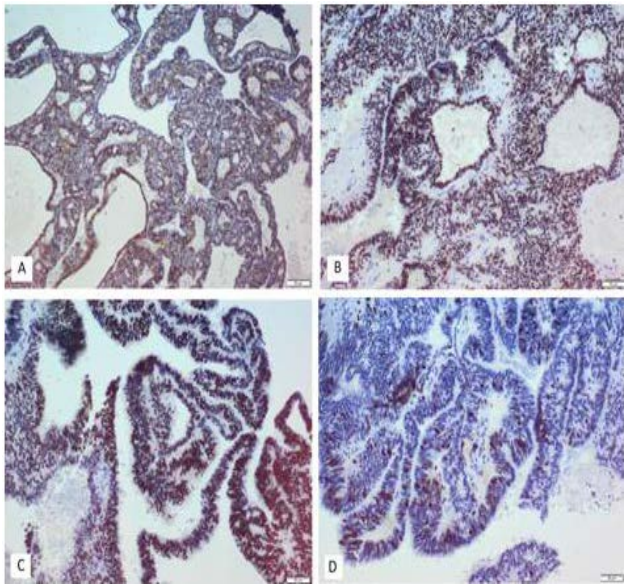

Fig. 2 – Immunophenotype of the tumor: A – GFAP, 40×; B – ER, 100×; C – PR, 100×; D – Ki67, 100×. GFAP – glial fibrillary acidic protein; ER – estrogen receptor; PR – progesteron receptor.

Discussion

Typical ependymomas behave in an indolent, slow-growing manner. While generally behaving in a benign clinical fashion, they possess capacity for localized tumor recurrence and tumor dissemination trough cerebrospinal fluid with metastases has been known to occur [5]. EnE appears to behave in a similar fashion, but there is a potential for malignant clinical behavior, although it appears to be an uncommon occurrence [5]. In presented case, onset of the disease was 14 years ago, and since that time, the patient has had multiple and extensive surgical procedures, due to metastatic spread.

While sacrococcygeal ependymomas are equally distributed among males and females, paraovarian pelvic and extra pelvic ependymomas have been exclusively reported in women, mainly of child bearing age [1, 3]. However, there was a case of a 75-year-old patient with pelvic EnE [5]. This reported gender and age related predominance is supported by numerous case reports, and is in accordance with our patient's gender and age. Initially, her disease was diagnosed when she was 27 years old, but at that time, the diagnosis was malignant mesothelioma. Since we do not have medical reports and histological slides, we can only doubt that it was also an EnE. Similar case was described by Verdun and Owen [5]: a female patient operated for upper right quadrant tumor, presumed to be metastatic pancreatic tumor, but postoperatively diagnosed as mesothelioma. Two years later, the patients developed recurrent tumor that was localized in pelvic region. At that time, tumor samples were reviewed and with the aid of immunohistochemistry, the diagnosis was corrected to EnE.

Ependimomas of CNS have distinct histology characterized by perivascular pseudorosettes, true ependymal rosettes, and fibrillary areas. In contrast, primary EnE have been described as having a wider range of microscopic architecture: perivascular pseudorosettes and occasionally true ependymal rosettes, along with mixtures of solid,

macrocystic, microcystic, pseudopapillary, papillary, trabecular, and cribriform architectures [3].

It is observed that EnE and ependimomas of CNS beside different clinicopathologic features, differ in immunophenotypical features as well. It may point to either a derivation from different precursors or differentiation along different pathways [6]. Different origin and development, immunophenotipical features and highly variable histology of EnE is at the same time a cause of many diagnostic errors [4].

The EnE are thought to be derived from embryonal rests in the paracoccygeal area, but there is also hypothesis that the pelvic ones might be arising from ectopic glial cells or might be neometaplasia of the peritoneal mesenchymal tissue [1, 5]. Another hypothesis proposes that some extraspinal ependymomas arise from germ cells and germ cell tumors (teratomas) and may therefore account for ependymomas arising in the ovary, parametrium, and anterior mediastinum [6, 7].

Idowu et al. [6] studied and compared primary CNS ependymomas EnE. Although both types stain positive for GFAP, they found several immunohistochemical differences, with EnE most likely positive for cytokeratins: 34betaE12 (60% vs. 0%), CK18 (100% vs. 20%), CAM 5.2 (60% vs. 10%), CK7 (80% vs. 10%). ER and PR are also strongly and diffusely positive in a majority of EnE compared with CNS ependymomas (ER: 100% vs. 10%; PR: 80% vs. 20%, respectively) that show weak and focal staining for these receptors in a minority of cases. CD99 and S100 are more commonly positive in CNS ependymomas than in their extraneural counterparts. This immunophenotype stated by Idowu et al. [6] is present in all published cases of EnEs [3–5], and it confirms the hypothesis that EnEs arise by different mechanisms from their CNS counterparts [6]. However, it appears that EnEs arising in different place or mechanism, show different immunoprofile. A case of ependymoma arising from mature cystic teratoma was published by Stolnicu et al. [4] and it showed immunofenotype of EnE, but it was markedly different from other EnEs.

In our case, ovaries were affected by the disease, and on one occasion even diagnosed as AFP positive germ cell tumor. Similar case was presented by Garcia-Barriola et al. [8], when a 30-year-old woman was given an erroneous diagnosis of poorly differentiated carcinoma of the ovary. The patient presented pelvic pain for one year prior to surgery. A second laparotomy revealed a bilateral pure ovarian ependymoma that infiltrated the uterus and presented implants on the omentum. Differential diagnosis included mainly endometrioid and small cell carcinoma of the ovary, but presence of typical ependymal rosettes and positivity to GFAP confirmed the diagnosis of ependymoma [8]. Data on AFP expression in EnE are rare, precisely we have found only one case mentioning negative AFP expression in pelvic EnE [4]. This may be the cause for debate on origin of the primary tumor, but it also emphasizes the importance of comprehensive and thorough differential diagnostic in cases of ovarian lesions. Chances of misdiagnosing EnEs of ovarian localization are high, particulary since they may show papillary areas with psammoma bodies, pseudofollicles, trabeculae, microcysts and other patterns resembling *struma ovarii*, granulose cell, Sertoli-Leydig cell, serous and Wolffian tumors [4, 9]. Therefore, diagnoses made at the age of 29 (cyst with granulosa cells) and the age of 38 (metastatic germ cell tumor) could be questioned.

We were able to do some additional immunohistochemistry on the tissue sample diagnosed as AFP positive germ cell tumor metastasis, so we applied GFAP staining. Tumor tissue showed moderate GFAP positivity, which confirmed our suspicions regarding the previous diagnosis. Indeed, it was a metastasis of primary, AFP positive EnE.

Besides differentiating primary neural from EnE, metastatic carcinoids and primitive neuroectodermal tumor should be considered as differential. As EnEs can appear in liver, lung, small intestine, omentum and even endometrium, this entity should also be kept in mind in the differential diagnosis of a primary or metastatic carcinoma with papillary or pseudopapillary structures. Although our case was not positive with any of the keratin antibodies, it should be borne in mind that ependymomas may express cytokeratins and may be misdiagnosed as metastatic carcinoma. In such cases if the morphologic pattern raises suspicion to EnE, GFAP staining will be a helpful diagnostic tool [1, 10].

Conclusion

This case demonstrates not only diagnostic immunophenotype of EnE, but above all an important principle in tumor pathology. Neoplasms may rarely occur in unusual and unexpected primary and metastatic sites. Pathologists need to be familiar with histologic features of a wide range of neoplasms and not just the appearance of neoplasms within their own limited subspecialty area. Correct diagnosis may be achieved by tumor pattern recognition on initial routine slides followed up by confirmatory immunostains.

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CASE REPORT
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Coronary embolism causing myocardial infarction after heart valve surgery

Infarkt miokarda izazvan koronarnom embolijom nakon operacije srčanih zalistaka

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Abstract

Introduction. Coronary embolism can rarely be a cause of myocardial infarction. It is usually associated with atrial fibrillation, dilated cardiomyopathy, bacterial endocarditis and underlying hypercoagulable state, as well as heart surgery. **Case report.** We reported a case of a patient with severe mitral and tricuspid regurgitation, with no underlying coronary artery disease. The patient underwent heart valve surgery, and the immediate postoperative course was uneventful. Five days after the operation, the patient sustained cardiac arrest, which was followed by a successful cardiopulmonary resuscitation. Electrocardiography showed atrial fibrillation with a significant ST segment elevation in the inferior leads. Urgent coronary angiography revealed a total occlusion of the right coronary artery, thus percutaneous coronary intervention was performed, after which flow restoration through the artery was achieved. The patient was discharged with triple antithrombotic therapy on the 20th postoperative day. **Conclusion.** Heart surgery could be followed by unexpected and potentially fatal complications, coronary embolism being one of them. In such case, the prompt and adequate reaction by the whole medical team is crucial for a patient's survival and recovery.

Key words:
coronary vessels; embolism; heart valves diseases; myocardial infarction; postoperative complications; resuscitation; treatment outcome.

Apstrakt

Uvod. Koronarna embolija je redak uzrok infarkta miokarda. Najčešće se povezuje sa atrijalnom fibrilacijom, dilatativnom kardiomiopatijom, bakterijskim endokarditisom i hiperkoagulabilnim stanjem, kao i sa operacijom srca. **Prikaz bolesnika.** Prikazan je slučaj bolesnika sa teškom mitralnom i trikuspidnom regurgitacijom, bez postojeće koronarne bolesti. Urađena je operacija srčanih zalistaka, a neposredni postoperativni tok je protekao uredno. Pet dana posle operacije došlo je srčanog zastoja koji je bio praćen uspešnim reanimacionim postupkom. Elektrokardiografski je registrovana atrijalna fibrilacija sa značajnom elevacijom ST segmenta u inferiornim odvodima. Urgentna koronarna angiografija je pokazala totalnu okluziju desne koronarne arterije, te se pristupilo perkutanoj koronarnoj intervenciji kojom je uspešno uspostavljen ponovni protok kroz arteriju. Bolesnik je otpušten 20. postoperativnog dana, sa trojnom antitrombotskom terapijom. **Zaključak.** Operacija srca može biti praćena neočekivanim i potencijalno fatalnim komplikacijama, među kojima je i koronarna embolija. Brza i adekvatna reakcija medicinskog tima ključna je za preživljavanje i oporavak bolesnika.

Ključne reči:
koronarni krvni sudovi; embolija; zalisci srca, bolesti; infarkt miokarda; postoperative komplikacije; reanimacija; lečenje, ishod.

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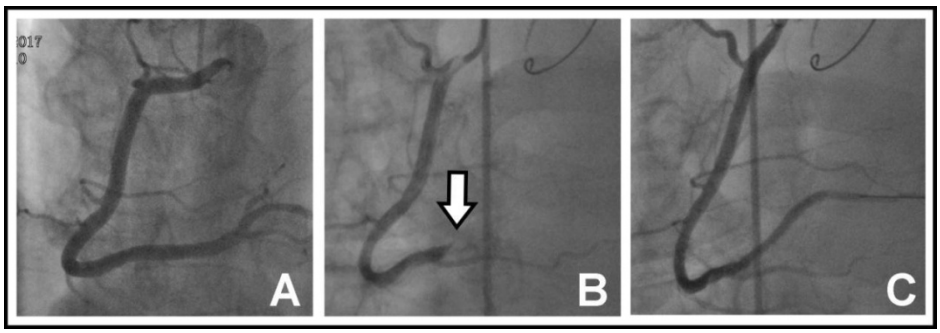

Fig. 1 – Coronary angiography: A) prior to the surgery: normal right coronary artery; B) after the cardiopulmonary resuscitation (CPR) following cardiac arrest: occlusion of the right coronary artery (arrow); C) after the percutaneous coronary intervention (PCI): recanalized right coronary artery.

Introduction

Coronary embolism (CE) is a rare nonatherosclerotic cause of myocardial infarction (MI). In an autopsy study performed decades ago, CE was accountable for 13% of MI [1]. A more recent clinical study showed even lower prevalence of CE in MI patients of only 2.9% [2].

The conditions associated with CE are atrial fibrillation (AF), dilated cardiomyopathy, bacterial endocarditis and underlying hypercoagulable state, as well as recent heart surgery [2, 3].

There is controversy regarding CE treatment. Different reperfusion strategies have all shown moderate success. Historically, drug therapy with anticoagulant and fibrinolytic agents was used. Nevertheless, percutaneous techniques such as percutaneous coronary intervention (PCI) thrombus aspiration, balloon angioplasty and stent implantation, have become preferred choice [4–6]. Yet, consensus was not reached regarding recommendations for the optimal treatment.

The significance of CE lies in the fact that, despite the low incidence, it represents an urgent, potentially fatal condition, with worse prognosis when comparing to MI caused by atherosclerosis [2].

Case report

We reported a case of a 61-year-old male who was admitted for elective surgery of heart valves. The patient complained of fatigue and shortness of breath. His heart rhythm was permanent AF, for which he was on oral anticoagulation therapy. The patient had a history of hypertension and hyperlipidemia, without other comorbidities. He was not a smoker and his family history was negative.

Echocardiography showed a severe mitral regurgitation (MR) with dilated mitral annulus (44 mm) and sclerotic lesions of mitral cusps and subvalvular apparatus. Mitral regurgitation (MR) effective orifice area was 40 mm^2 and regurgitant volume was 70 mL. Left atrium was extremely dilated with 57 mm in diameter and 142 mL in volume (indexed volume 69 mL/m^2). Left ventricular ejection function was preserved (60%), however diastolic dysfunction type I (impaired relaxation) was present (E/E' = 11). There was a moderate tricuspid regurgitation with a moderate pulmonary hypertension.

Coronary angiography prior to surgery excluded coronary artery disease (Figure 1A). The patient was afterwards referred to a cardiac surgeon for mitral and tricuspid valve surgery.

The patient underwent surgery in general anesthesia with the use of extracorporeal circulation. Mitral annuloplasty with the implantation of a rigid ring No. 30 and De Vega tricuspid annuloplasty were performed. The operation was successfully completed and the immediate postoperative course was uneventful.

Postoperative electrocardiography (ECG) showed AF with a normal ventricular rate (Figure 2), there were no changes in comparison to the preoperative ECG.

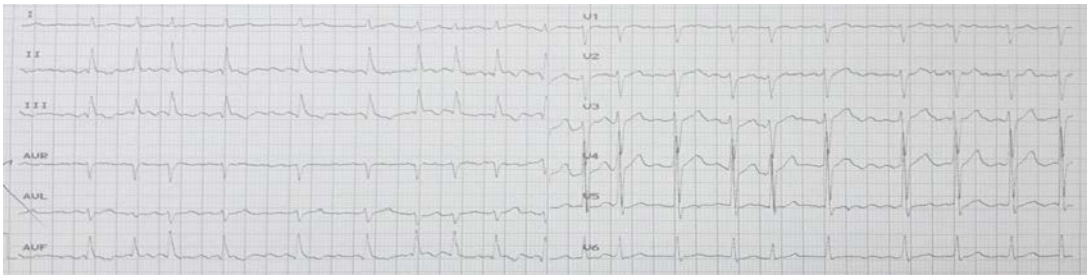

Fig. 2 – Postoperative electrocardiographic finding (ECG): atrial fibrillation with normal ventricular response and without any ST segment abnormalities.

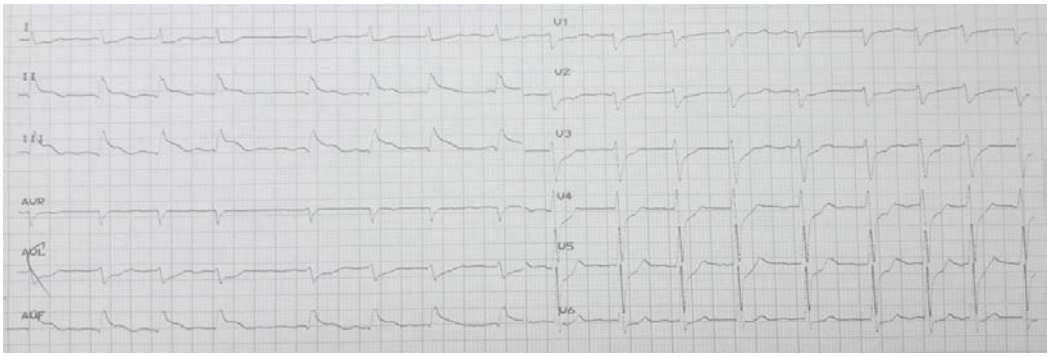

Fig. 3 – Electrocardiographic finding after the cardiopulmonary resuscitation: AF with elevation of ST segment in the inferior leads.

Anticoagulation therapy with low-molecular weight heparin was initiated, followed by a graduate introduction of warfarin after the removal of chest tubes on the 2nd postoperative day, with a starting dose of 2.5 mg/day and a gradual increase to 5 mg/day.

On the 5th postoperative day, the patient complained of a severe chest pain with a sudden onset, which was followed by a cardiac arrest due to ventricular fibrillation. The cardiopulmonary resuscitation (CPR) was immediately initiated. The patient was intubated and mechanically ventilated, and after ten attempts of defibrillation, along with medical support, return of spontaneous circulation was achieved.

The laboratory analyses at the moment of the incident showed INR 1.2, despite the target values being set to 2.0–3.0, and a significant increase in cardiac enzymes levels: creatine kinase 1,471 U/L, creatine kinase MB (CKMB)154 U/L, high-sensitive troponin T > 40,000 ng/L and D-dimer > 10,000 mg/mL.

ECG showed AF with a significant ST segment elevation in the inferior leads (Figure 3). The patient was in cardiogenic shock with mean arterial pressure of 50 mmHg and heart rate of 130 bpm, thus noradrenaline infusion was administered. Arterial blood gases showed metabolic acidosis: pH 7.11, lactate 10 mmol/L, basae excess (BE) -18.5 mmol/L, pO_2 47 mm Hg, pCO_2 31 mm Hg, SaO_2 65% (nr > 94%)].

Dual antiplatelet therapy was immediately initiated, including aspirin and clopidogrel (loading doses 300 mg and 600 mg, respectively, followed by maintenance doses 100 mg/day and 75 mg/day, respectively). Urgent coronary angiography revealed a total occlusion of the distal right coronary artery (RCA) with thrombolysis in myocardial infarction (TIMI) 0 flow (Figure 1B). Primary PCI was indicated. After an initial balloon dilatation which did not result in restoration of TIMI flow, multiple thrombi aspirations were performed. However, due to distal embolization, aspiration catheter could not be placed deep into the postero-lateral branch of the RCA, so the whole thrombi could be aspirated. Thus, downstream abciximab was given, intravenous (iv.) bolus of 0.25 mg/kg followed by an infusion of 0.125 μg/kg/min for 12 h. Furthermore, due to the large thrombus burden, two drug-eluting stents were implanted resulting in optimal outcome and TIMI 3 flow restoration at the end of the procedure (represented normal epicardial reperfusion) (Figure 1C).

After the successful PCI, the patient was returned to the intensive care unit. Repeated echocardiogram showed normal function of the mitral and tricuspid valves, but akinesia of the inferior wall was registered. Despite the timely performed PCI, mechanical ventilation and noradrenalin infusion, the patient was still hypotensive and hypoxic, which, along with extremely elevated D-dimer level, raised a suspicion for pulmonary embolism. However, it was excluded by computerized tomography pulmonary angiography.

The ECG changes gradually resigned. The intensive care treatment resulted in hemodynamic stabilization and oxygen level restoration, so after five days the patient was extubated and measures of early rehabilitation were initiated.

Further course was uneventful. The warfarin dose was increased to the maximal 10 mg/day in order to reach the therapeutic INR value. A 24 h monitoring of the ECG recorded AF with a good heart rate control. The patient was discharged on the 20th postoperative day in a good general condition with triple antithrombotic therapy including aspirin, clopidogrel and warfarin.

Three months following discharge, the patient was asymptomatic with no signs of heart failure [New York Heart Association (NYHA) class I and Canadian Cardiology Society (CCS) grade I]. ECG showed AF with a good rate control and no signs of myocardial ischemia and lesion. Echocardiography revealed normal function of the mitral and tricuspid valves with normal systolic function of the left ventricle and no wall motion abnormalities.

Discussion

The unexpected life-threatening event in the postoperative course of our patient was evidentially caused by MI. This was confirmed by the ECG which showed ST segment elevation in the inferior leads, as well as the elevated levels of cardiac enzymes in the blood and the total occlusion of RCA seen by coronary angiography.

MI due to coronary artery injury during heart valve surgery is a rare but possible complication. Because of the

Table 1

Criteria for the diagnosis of coronary embolism [2]

Major criteria
• Angiographic evidence of coronary artery embolism and thrombosis without atherosclerotic components • Concomitant coronary artery embolization at multiple sites • Concomitant systemic embolization without left ventricular thrombus due to acute myocardial infarction
Minor criteria
• < 25% stenosis on coronary angiography, except for the culprit lesion • Evidence of an embolic source based on transthoracic echocardiography, transesophageal echocardiography, computed tomography, or magnetic resonance imaging • Presence of embolic risk factors: atrial fibrillation, cardiomyopathy, rheumatic valve disease, prosthetic heart valve, patent foramen ovale, atrial septal defect, history of cardiac surgery, infective endocarditis, or hypercoagulable state
Definite diagnosis of coronary embolism
• Two or more major criteria, or • One major criterion plus two or more minor criteria, or • Three minor criteria

close proximity of the mitral annulus with the left circumflex coronary artery, this artery can be injured during mitral valve surgery [7, 8]. For the same reason, tricuspid valve surgery caries a risk of the right coronary artery injury [9, 10]. There are multiple mechanisms for these injuries, but the majority include mechanical obstructions caused by a surgical needle or a suture [8, 11, 12].

In all the published cases, MI occurred during the operation or in the following few hours. Considering that in our case more than 96 hours passed since the end of the operation and that the patient was completely stable during this time, without any ECG or laboratory abnormalities, this mechanism was excluded as the cause of the MI.

The patient had AF and the oral anticoagulant therapy was administered after the operation. The INR values, however, did not yet reach the optimal therapeutic range. For this reason, CE was proposed as an underlying cause of MI. AF could have led to a thrombus formation in the extremely dilated left atrium, which then embolized to the coronary artery through the normal anatomic path. The thrombi aspirations during the PCI increased the suspicion for this mechanism. Clinical studies addressing this topic have shown that AF is the most common underlying cause of CE,[2, 3] which supports this theory.

A similar case was reported by Wang et al. [13] in which MI occurred seven weeks after heart surgery in a patient with prior history of AF. In this case, a definitive diagnosis of CE was established after echocardiographic finding of a thrombus in the left atrium. The INR value at the moment of the incident was subtherapeutic, like in our case. Because this was a patient with permanent AF and acute MI, triple antithrombotic therapy was recommended for the first 6 months after discharge from the hospital. After that period aspirin or clopidogrel could be excluded, and after 12 months, if no major adverse cardiac events occur, single therapy with warfarin could be considered.

None of the eminent cardiovascular societies have published clinical guidelines concerning diagnosis and treatment of CE. Shibata et al. [2] suggested the criteria useful for the CE diagnosis (Table 1). Our patient had one major criterion: angiographic evidence of coronary artery thrombosis. Minor criteria present were: < 25% luminal stenosis on other coronary arteries except for the culprit lesion, and presence of the risk factors for thromboembolism, such as AF, cardiomyopathy and recent cardiac surgery. Presence of one major and two minor criteria are sufficient for the diagnosis of CE.

MI can be classified as the type 2 according to the universal classification of MI [14]. This stands for MI secondary to an ischemic imbalance, which includes CE. MI cannot be interpreted as a surgical complication, considering that more than 72 hours passed between the operation and the event, and that during this time the patient was completely stable and had normal recovery.

Conclusion

Heart surgery could be followed by a wide range of complications and although a large number of them are well known and predictable, there are some that are rare and unexpected but potentially fatal, coronary embolism being one of them. The prompt and adequate reaction by the whole medical team in such case is crucial for a patient's survival and recovery.

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CASE REPORT
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Recurrent painful ophthalmoplegic neuropathy: A report on the patient from the Romani population and 82-year-old patient

Rekurentna bolna oftalmoplagička neuropatija – prikaz bolesnika iz romske populacije i 82-godišnjeg bolesnika

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Abstract

Introduction. The current diagnostic criteria for recurrent painful ophthalmoplegic neuropathy (RPON) are at least two attacks of unilateral headache, associated with ipsilateral paresis of one, two or all three cranial nerves (III, IV or VI). There is no case report about RPON in the Romany population. The oldest patient with RPON, published in the literature, was 74 years old. **Case report.** The first patient was a 31-year-old man from the Romani population who was treated during three episodes of RPON, with III nerve palsy during one episode and with alternating VI nerve palsy during two episodes. All examination were normal except serum lipid levels and Cytomegalovirus immunoglobulin G (CMV IgG), *Toxoplasma gondii* IgG, Epstein–Barr virus (EVB) IgG and Varicella zoster IgG which were elevated. The second patient was a 82-year-old male patient with two RPON episodes with alternating VI nerve palsy. All examinations were normal, except Herpes simplex type 1 virus IgG, CMV IgG, *Toxoplasma gondii* IgG, EBV IgG and Varicella zoster IgG which were elevated, and his brain magnetic resonance imaging (MRI) showed lacunar ischemic lesions. Both patients were started on corticosteroid. Recovery was completed after all five episodes of RPON. **Conclusion.** There are no data on the frequency of RPON among the Romani population. The presentation of RPON in the oldest age is rare. RPON should be considered as a diagnostic option in these minorities. New case reports or systematic review articles about RPON are necessary to create a new insight into the nature of the disease.

Key words:
ophtalmologic migraine; adult; aged, 80 and over; diagnosis; drug therapy; roma; treatment outcome.

Apstrakt

Uvod. Aktuelni dijagnostički kriterijumi za rekurentnu bolnu oftalmoplegičnu neuropatiju (RPON) su najmanje dva ataka jednostrane glavobolje, udružene sa ipsilateralnom parezom jednog, dva ili sva tri kranijalna nerva (III, IV ili VI). Do sada nije prikazan slučaj RPON u populaciji Roma. Najstariji bolesnik sa RPON, prikazan u literaturi, imao je 74 godine. **Prikaz bolesnika.** Prvi bolesnik je bio 31-godišnji muškarac romske nacionalnosti koji je lečen tokom tri epizode RPON, sa parezom III kranijalnog nerva u tokom jedne epizode i VI kranijalnog nerva na različitim stranama, u toku druge dve epizode. Sva ispitivanja su bila uredna, osim povišenih vrednosti lipida u serumu i imunoglobulina G (IgG) na citomegalovirus (CMV IgG), IgG na toksoplazmu gondi, IgG na Epstein–Barr virus (EVB) i IgG na Varicela zoster virus. Drugi bolesnik je bio 82-godišnji muškarac sa dve epizode RPON i zahvećenim VI kranijalnim nervom na različitim stranama. Sva ispitivanja su bila uredna, osim povišenih vrednosti IgG na Herpes simplex virus tip 1, CMV IgG, IgG na toksoplazmu gondi, EBV IgG i IgG na Varicela zoster virus i lakunarnih ishemijskih lezija koje je imao na snimku magnetne rezonance (MR) mozga. Oba bolesnika su lečena kortikosteroidnom terapijom. Oporavak je bio kompletan nakon svih pet epizoda RPON. **Zaključak.** Ne postoje podaci o učestalosti RPON među pripadnicima romske nacionanosti. Pojava RPON u najstarijem životnom dobu je veoma retka. RPON bi trebalo imati u vidu kao jednu od dijagnostičkih opcija kod ovih grupa bolesnika. Novi prikazi bolesnika ili revijalni radovi o RPON su neophodni da bi se razjasnila priroda same bolesti.

Ključne reči:
migrena, oftalmoplegična; odrasle osobe; stare osobe, 80 i više godina; dijagnoza; lečenje lekovima; romi; lečenje, ishod.

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Introduction

Recurrent painful ophthalmoplegic neuropathy (RPON) is new concept from the 3rd edition of the International Classification of Headache Disorders (ICHD-3) and classified under the category of painful cranial neuropathies and other facial pains [1] which according to previous classification from 2004 marked as ophthalmoplegic migraine (OM) and classified under the category of cranial neuralgias [2].

The current diagnostic criteria for RPON are at least two attacks of unilateral headache, associated with ipsilateral paresis of one, two or all three cranial nerves (III, IV or VI). Orbital and parasellar and posterior fossa pathological lesions must be excluded by appropriate diagnostic techniques as well as other potential diagnoses according to the ICHD-3 criteria [1].

There is no case report about RPON in the Romani population. There is only one study about prevalence of migraine and risk factor for migraine in the Romani population from Spain. Prevalence of migraines is greater in the Romani living in Spain than in the general Spanish population [3].

The oldest patient with RPON, published in the literature so far, was 74 years old [4].

RPON is diagnosis of exclusion. Possible dilemma that should not be overlooked is Tolosa-Hunt syndrome. Diagnostic criteria for Tolosa-Hunt syndrome are granulomatous inflammation of the cavernous sinus, superior orbital fissure or orbit, demonstrated by magnetic resonance imaging (MRI) or biopsy [5]. Other diagnostic dilemmas are orbital myositis, neoplastic disease, vascular disease, brain stem ischemia, mass or multiple sclerosis lesions, diabetic palsy, traumatic nerve palsy, infection, myasthenia gravis, Miller Fisher syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), idiopathic intracranial hypertension or hypotension, thyroid ophthalmopathy, orbital mass, narrow angle glaucoma, Wegener's granulomatosis, vincristine therapy [1, 6–11].

The RPON is rare entity whose pathophysiology is unknown and for which no therapeutic recommendations are available [11].

Case reports

Case 1

A 31-year-old man from the Romani population was admitted to the Clinic of Neurology, Faculty of Medical Sciences, University of Kragujevac, during the third RPON episode which began in 2014, as left side periorbital headache, 7/10 in intensity, pulsating in character, associated with nausea. A few hours after, the patient developed left sided VI cranial nerve palsy and diplopia. He was started on prednisolone 120 mg for 10 days, after that 80 mg for 10 days, followed by 60 mg for 10 days. The second day of the disease, headache intensity was reduced. At the discharge, the patient was without headache and with mild recovery of the mobility of the eyeball. After 3 months of the onset of symptoms, patients was completely recovered.

During hospitalization his general examination was normal. Nervous system examination showed left sided VI nerve palsy. All laboratory results were normal except serum lipid levels which were high. Blood glucose levela and hemoglobin A1c (HbA1c) levels were normal. Immunological analysis, thyroid hormones and antibodies to thyroid hormones were normal. Virological, bacteriological and parasitological analyses were normal. Brain MRI with contrast, MR angiography of the brain arteries, chest X-ray and visually evoked potentials (VEP), all were normal. We excluded neuromuscular junction disorders by prostigmin test. Cerebrospinal fluid (CSF) analysis was normal [cell counts, protein, glucose levels, imunoglobulin G (IgG) index].

The first episode occurred in 2010 with right-sided headache, 8/10 in intensity, nausea, vomiting, photophobia and diplopia. On neurological examination, the patient had isolated right VI nerve palsy. We applied corticosteroid therapy. The headache lasted 6 months, while the double vision were present 7 months after the onset of symptoms. All results were normal.

The second episode occurred in 2013, with right-sided occipital headache, 9/10 in intensity, nausea and vomiting. A few days later, the patient developed diplopia. On neurological examination, he had isolated right III nerve palsy. During hospitalization, the patient developed right ptosis. The reaction of the pupil to light and accommodation were normal, bilaterally. Headache was stopped within 3 days from administration of corticosteroid therapy while diplopia lasted for about 2 months. All results were normal.

Before the onset of RPON, the patient was healthy and had never suffered from headaches. The mother of the patient had a migraine in generative period of life.

Case 2

A 82-year-old male patient was admitted in our Clinic during the second RPON episode which began in 2017 with a pain in the depth of the right eye and diplopia. Seven days later, the patient developed the right VI nerve paralysis, and other neurological findings were normal. Increased cholesterol levels were found in laboratory analyses. Blood glucose level and HbA1c level were normal. Immunological analysis, thyroid hormones and antibodies to thyroid hormones were normal. Virological, bacteriological and parasitological analyses were normal. The test for diplopia showed diplopia in the direction of left lateral rectus muscle. The ophthalmologic examination showed the initial cataract. Lacunar ischemic lesions in white matter of parieto-occipital region on the brain MRI were seen. MR angiography of the brain arteries, visually evoked potentials (VEP), echo tomography of the orbits, eye pressure, and Doppler ultrasonography of the blood vessels of the neck – all were normal. We excluded neuromuscular junction disorders by prostigmin test. We applied dexamethasone and acetylsalicylic acid for 7 days. Ophthalmological rehabilitation was conducted. Three

months later there was a complete recovery. A diagnosis of RPON was made.

The first episode of RPON occurred in 2015 due to left side orbital pain, 6/10 in intensity, nausea and diplopia. In the neurological examination, the patient had isolated left VI nerve palsy. The same therapy was administered. After 3 months of the onset of symptoms, patients was completely recovered.

Before the onset of RPON, the patient was healthy and had never suffered from headaches.

Discussion

RPON is a very rare disease, with an incidence of 0.7 per million [12]. The incidence of RPON is two times higher in female, according to some studies [4, 13, 14], whereas in one case series ratio of men to women is approximately 1: 1 [15].

The side of headache and nerve palsy is the same in the largest number of cases during different attacks of RPON [14], while the change of side is very rare (Gelfand et al. [4] found only two cases in their series of 84 patients). Alternating side of VI nerve palsy during different RPON episodes was described in a few cases [15, 16] like alternating side of III nerve palsy [15].

Only 1–6.5% of patients had symptoms on different side in two different attacks like our first patient [4, 15].

The most of patients (up to 94%) had normal CSF findings [4] and only few had nonspecific CSF abnormalities [15].

The brain MRI in 75% of patients with III nerve palsy shows an accumulation of gadolinium in the affected nerve area during the attack of the disease, but in remaining cases, the brain MRI is normal [4, 6–8, 14, 15, 17].

Our second patients had VI nerve palsy in two episodes of RPON, on different side, without changes on the brain MRI. The brain MRI [7, 15, 17] and brain single photon emission computed tomography (SPECT) [18] show no lesions in the most patients with VI nerve palsy, although there have been cases presented with the enhancement of intraparenchymal and cisternal part of VI nerve [9, 18]. In our patients, after three and two attacks of RPON, the symptoms resolved completely in a period of several weeks to several months, such as the recently published case of RPON [19].

Recently, a report on recurrent Tolosa-Hunt syndrome in patients with and without granulomatous changes in the brain MRI has been published [5]. Since, it is virtually impossible to distinguish patients with recurrent Tolosa-Hunt syndrome without a change in the brain MRI from patients with RPON, and at a time when biopsy is practically very rarely performed and in the era of modern high-quality neuroradiology diagnostics, the brain MRI remains a key factor for diagnosis of RPON and Tolosa-Hunt syndrome. It is extremely important to strictly follow the brain MRI criteria for diagnosis of Tolosa-Hunt syndrome (granulomatous inflammation of the cavernous sinus, superior orbital fissure or orbit, demonstrated by the brain MRI or biopsy) and the brain MRI criteria for RPON (orbital and parasellar and posterior fossa pathological lesions must be excluded by appropriate diagnostic techniques). An excellent response to corticosteroid therapy for RPON and Tolosa-Hunt syndrome patients eliminates the therapeutic dilemma.

However, new case reports or systematic review articles about RPON are necessary to create a new insight into the nature of the disease.

Conclusion

Due to the excellent response to corticosteroid therapy in RPON and Tolosa-Hunt syndrome and due to the same differential diagnosis, we consider that the detailed examination should be conducted in all patients with suspicion on RPON/Tolosa-Hunt and corticosteroid therapy should be administered. Also, it is necessary to make a more detailed distinction between the definitions of clinical picture between these two diseases in new headache classification.

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COVID-19 infection in patients with malignant diseases

COVID-19 infekcija kod onkoloških bolesnika

To the Editor:

A series of cases with pneumonia of unknown cause emerged in December, 2019 in Wuhan, Hubei, China. The clinical presentations greatly implied that it could be pneumonia of viral origin [1]. A total of 201 cases of pneumonia in China have been confirmed. On January 3, 2020, the Chinese scientists identified the first complete genome of the novel β genus coronaviruses (2019-nCoVs) in samples of bronchoalveolar lavage fluid (BALF) from a patient from Wuhan. Three distinct strains have been identified, the virus has been designated as 2019-nCoV [2], and infection caused by this virus as COVID-19 (Coronavirus disease-19).

Patients with cancer are more susceptible to infections than individuals without cancer because of their systemic immunosuppressive state caused by the malignancy itself and anticancer treatments, such as chemotherapy or surgery [3–5]. One of the first reported cohort of COVID-19 patients, showed that among them there was 1% of cancer patients. Lung cancer was the most frequent type being present in 5 of 18 patients (28%) [6].

In this study we present preliminary data of oncology patients found positive for COVID-19 within first 6 months of pandemic. All patients were treated in the University Clinical Center of the Republic of Srpska in Banja Luka. Main inclusion criteria were patients with diagnosis of any form of malignanat neoplasma (solid tumors and haematologic malignancies) with the COVID-19 diagnosis, laboratory confirmed using real time-polymerase chain reaction test (RT-PCR) test. We evaluated characteristics of the patients and diseases both for cancers and for COVID-19, as well as the correlation between potential prognostic factors and outcome.

Totally, 66 patients with cancer had a positive RT-PCR test for COVID-19. In average, patients had 67 years; there were 46 (69.70%) male and 20 (30.30%) female patients. The most common were hematological malignancies [n = 14 (21.2%)], gastrointestinal cancers [n = 12 (18.2%)], lung cancers [n = 9 (13.6%)], urological cancers [9 (13.6%)], breast cancer [7 (10.6%)], etc. There was 10 (15.20%) of the patients who received radiotherapy and 25 (37.90%) of the patients who recived chemotherapy within one month before COVID-19. Most of the patients were in the stage IV disease [32 (50.80%)], three of them were not available for staging evaluation and 13 (20.60%) of them were cancer survivors. Most of the patients (37.90%) had the Eastern Cooperative Oncology Group (ECOG) perforamance status 0. Severe complications of infection developed in 8 (12.10%) of the patients, moderate clinical course had 19 (28.80%) patients and mild clinical course had 39 (59.10%) of the patients. Sixteen (24.20%) of the patients died, others recovered from the virus infection. Data on post COVID-19 survivors (50 patients) were as follows: for 4 of them there were no data, 4 (8%) of the patients died two month after the infection, others were alive at the moment of data analysis. Thirty seven of 66 patients (56.10%) had cardiovascular comorbidity, diabetes melitus had 11 (16.7%) of the patients, 17 (25.80) of the patients had multiple comorbidities; no comorbidities had 19 (28.80) of the patients. Bivariate Pearson's analisys showed signifficant correlation beetwen cardiovascular comorbidity and death ($p = 0.019$). Also, there was a statistically signifficant correlation between severity of clinical course and age of patients as well as lethal outcome ($p < 0.01$). Most of deaths ocurred in patients with haematological malignancy and colorectal cancer. Two of nine patients with lung cancers died, and one death each was registered in breast cancer, sarcoma, ginecological and brain cancer patients. Most of haematooncology patients were in active treatment and had stage IV of the disease. All death associated with colorectal cancer were in patients with active oncological tratment.

In this initial report, mortality among cancer patients was higher than in general populations of infected patients (24.20%). An early report of a subset of patients who died from COVID-19 in Italy found that 20.3% of the deceased had an active cancer. All of this underlines the increased risk for cancer patients, particularly lung cancer patients [7]. The TERAVOLT study showed that mortality rate in the thoracic cancer patients was 33% [8]. The Institute Gustave Roussy reported data on 137 COVID-19 oncology patients. After admission, 25% of the patients had worsening, 11% were

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admitted to an intensive care unit and 15% died. Hematooncology patients were more likely to have worse outcomes. In the continuation of the study, it was shown that treatment with chemotherapy within three months, but not targeted therapy or immunotherapy, doubled the chance of worsening disease [9]. According to our center experience, chemotherapy and radiotherapy did not affect survival in patients with COVID-19, although the number of patients was small for definitive analysis. It is important to notice that patients with colorectal cancer who died from COVID-19, were in the active treatment. Patients with haematological malignancies had most cases and most deaths. Also, we can not say that comorbidities such as cardiovascular diseases and multiple comorbidities appear as predictors for poor outcomes in our patient population, because they are associated with increased risk of death in the general population, too. We must also take into account that average age of our patients was 67, they had cancer diagnosis alone or associated with comorbidities, they continously were in a state of imunosupression either due to the cancer itself or due to specific oncological therapy.

In summary, cancer patients are at high risk of complications if infected with coronavirus, both directly and indirectly due to treatment interruption. We must provide the continuation of oncological treatments (surgical, chemotherapy, radiotherapy etc.) without delay.

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