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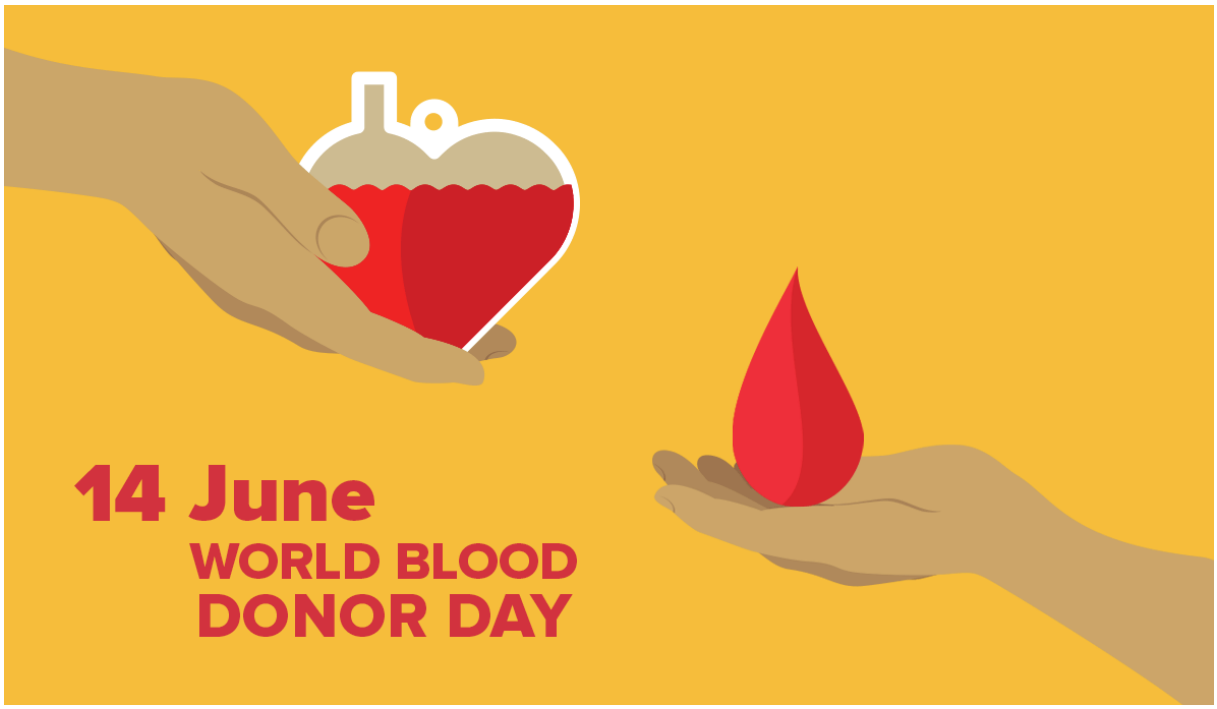

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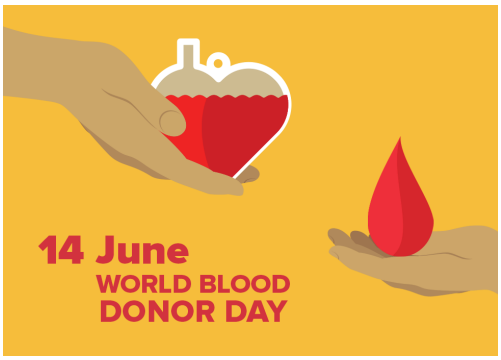

World Blood Donor Day is celebrated every year around the world on June 14th, the day of the Nobel Prize winning scientist Karl Landsteiner's birthday (14 June 1868), who discovered the ABO blood group system. This event marking was first started in the year 2004 with an aim to thank voluntary blood donors for their life-saving gift and to raise awareness of the significance for regular blood donation to ensure availability of blood and blood products for persons in need.

This year marks the 150th birthday of Karl Landsteiner.

Svetski dan dobrovoljnih davalaca krvi proslavlja se širom sveta 14. juna, na dan rođenja (14. jun 1868) Karla Landštajnera, dobitnika Nobelove nagrade za otkriće krvnih grupa. Obeležavanje ovog dana započeto je 2004. godine u znak zahvalnosti dobrovoljnim davaocima krvi za njihov poklon koji spasava život i sa ciljem podizanja svesti o značaju redovnog dobrovoljnog davanja krvi, čime se obezbeđuju krv i proizvodi od krvi svima kojima je to potrebno.

Ove godine navršava se 150 godina od rođenja Karla Landštajnera.

ORIGINAL ARTICLES

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Decreased ultrasound echogenicity as a thyroid hypofunction marker and correlation with autoantibody levels

Smanjena ultrazvučna ehogenost kao pokazatelj hipofunkcije štitaste žlezde i korelacija sa nivoima autoantitela

Dragan Dimić, Milena Velojić Golubović, Saša Radenković, Danijela Radojković, Milica Pešić

Clinical Center Niš, Clinic of Endocrinology, Niš, Serbia

Abstract

Background/Aim. The value of ultrasound in functional disorders can be significant. That is why the question arises on the use of ultrasound examination of thyroid gland and its echogenicity as a screening method in early detection of disfunctions, of the gland primarily subclinical and clinical forms of hypothyreoidism. The objective of this paper was to determine antibodies of thyroid peroxidase (anti-TPO) and thyroglobuline antibodies (anti-TG) increase frequency in relation to the character of ultrasound echogenicity as well as to estimate the frequency of subclinically and clinically obvious hypothyreoidism in relation to the changed echogenicity. **Methods.** Study included 656 patients in outpatient clinic during 2014. All examinees underwent ultrasound examination of thyroid gland, the blood was taken for determination of free thyroxine (FT4), thyroid-stimulating hormone (TSH), anti-TPO and anti-TG. The patients were divided into two groups; the group A with normal echogenicity of thyroid gland tissue, and the group B with decreased echogenicity. The group B was divided into two subgroups, B1 with a mildly decreased and B2 with significantly decreased echogenicity. **Results.** TPO antibody, TSH and TG antibody positivity and their mean values in the group B were significantly higher, as well as in subgroups B1 and B2, in relation to the group A ($p < 0.001$). In the group A, only 4 (1%) examinees were indicated with subclinical hypothyreoidism. In the group B, the sublinical hypothyreoidism was indicated in 42, while the clinical hypothyreoidism was indicated in 16 examinees. Fifty-eight (25%) examinees suffered from thyroid gland altered function. In the subgroup B1, 16 examinees were indicated with subclinical and 4 with clinical hypothyreoidism. Twenty (11%) examinees suffered from altered thyroid function. In the group B2, the subclinical hypothyreoidism was found in 26 examinees, while the clinical hypothyreoidism was found in 12. Thirty-eight (76%) examinees suffered from altered thyroid function. **Conclusion.** The ultrasound screening of thyroid gland plays an important role in early detection of thyroid disfunction, i.e., sublinical and clinical hypothyreoidism. Decreased ultrasound echogenicity represents the significant marker of altered thyroid gland function. In these persons we have determined the high percentage of subclinical and clinical hypothyreoidism frequency.

Key words:
thyroid gland; hypothyroidism; ultrasonography; thyroid hormones; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Vrednost ultrazvučnog pregleda kod poremećaja funkcije štitaste žlezde može biti velika. Zato se postavlja pitanje korišćenja ultrazvučnog pregleda kao skrining metode u ranom otkrivanju poremećaja funkcije žlezde, pre svega supkliničke i manifestne hipotireoze. Cilj ove studije bio je da utvrdi povećanje tiroid peroksidaza antitela (anti-TPO) i tireoglobulinskih antitela (anti-TG) u odnosu na ultrazvučne ehogenosti, kao i učestalost subkliničke i kliničke hipotireoze u odnosu na izmenjenu ehogenost. **Metode.** U ispitivanje je bilo uključeno 656 bolesnika tokom 2014. Kod svih ispitanika urađen je ultrazvučni pregled štitaste žlezde i određene vrednosti slobodnog tiroksina (TT4), tiroid-stimulišućeg hormona (TSH), anti-TPO i anti-TG. Ispitanici su bili podeljeni u dve grupe. Grupu A čine su osobe sa normalnom ehogenošću tkiva štitaste žlezde, a grupu B osobe sa smanjenom ehogenošću. Grupa B podeljena je bila u dve podgrupe: B1 sa umereno smanjenom ehogenošću i B2 sa značajno smanjenom ehogenošću. **Rezultati.** Srednje vrednosti TSH, anti-TPO i anti-TG bile su značajno više u grupi B, I podgrupama B1 i B2, u odnosu na grupu A ($p < 0.001$). Samo 4 (1%) ispitanika u grupi A su označena kao subklinička hipotireoza. U grupi B subklinička hipotireoza je dijagnostikovana kod 42 ispitanika, a hipotireoza kod 16 ispitanika, što znači da je 58 (25%) ispitanika imalo izmenjenu funkciju štitaste žlezde. U podgrupi B1 subklinička hipotireoza je nađena kod 16, a hipotireoza kod 4 ispitanika,

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odnosno njih 20 (11%) je imala izmenjenu funkciju žlezde. U podgrupi B2, supklinička hipotireoza nađena je kod 26, a hipotireoza kod 12 ispitanika, njih 38 (76%) imalo je izmenjenu funkciju štitaste žlezde. **Zaključak.** Ultrazvučni skrining štitaste žlezde ima važnu ulogu u ranoj detekciji izmenjene tireoidne funkcije, pre svega supkliničke i manifestne hipotireoze. Smanjena ultrazvučna ehogenost tireoidnog tkiva značajan je marker izmenjene funkcije. Kod ovih osoba našli smo značajno veću učestalost supkliničke i kliničke hipotireoze.

Ključne reči:
tireoidna žlezda; hipotireoidizam; ultrazvuk; tireoidna žlezda, hormoni; osetljivost i specifičnost.

Introduction

Ultrasonography represents very frequently used examination method in diagnostic procedure of various thyroid gland diseases. This method has practically no restrictions and it is available and relatively cheap. That is why it is applied in patients without pronounced symptoms of the disease, but also in those with uncharacteristic symptoms and signs like fatigue, dizziness, vertigo, irritation, anxiety, aggravated swallowing and breathing, irregular menstrual periods, heart rhythm alterations, blood pressure rise and plasma lipids level rise. These are sometimes the signs of some thyroid gland diseases.

Ultrasound method is a sovereign method in diagnostics of morphological thyroid gland changes. Its value in functional disorders diagnostics and diagnostics of inflammatory processes can also be significant. That is why the question arises on the use of ultrasound examination of thyroid gland and on the estimation of its echogenicity as a screening method in early detection of disfunctions, primarily subclinical and clinical forms of hypothyreoidism. Results of decreased thyroid gland tissue echogenicity and thyroid-stimulating hormone (TSH) level increase could be the early signs of thyroid gland function failure and the subclinical hypothyreoidism diagnosis, and are significant when having in mind its tendency to progress into the clinically manifested hypothyreoidism. Moreover, this examination method can be of significant assistance in discovering autoimmune thyroiditis (AT) where the fine-needle biopsy (FNB) diagnosis is recognized as a golden standard.

Decreased echogenicity of the thyroid gland tissue is considered specific ultrasound result in autoimmune thyroiditis, but also in hypofunction of the thyroid gland [1–4]. Accuracy and reliability of this kind of estimation are still not clear enough. The increase of antibodies level, first of all thyroid peroxidase (anti-TPO), represents the important parameter in diagnostics of these kinds of disorders [5]. Other irregularities in the ultrasound scans, like nodules with or without decrease of tissue echogenicity, can also be the signs of altered thyroid gland function [6].

The objective of this paper was to determine anti-TPO and thyroglobuline antibodies (anti-TG) increase frequency in relation to the character of ultrasound echogenicity as well as to estimate the frequency of subclinically and clinically obvious hypothyreoidism in relation to the changed echogenicity.

Methods

Study included 656 (622 female and 34 male) outpatients aged 17–68 years, examined at the specialized outpatient facility and at the Ultrasound Diagnostics Cabinet of the Polyclinic Department of the Clinic of Endocrinology, Clinical Center Niš, during 2014. The testing excluded persons already suffering from thyroid gland disfunction as well as persons with indicated increase of thyroid gland function in the course of our testing. All examinees underwent ultrasound examination of thyroid gland, the blood was taken for the lab analysis and for determination of free thyroxine values (FT4), TSH, to anti-TPO and anti-TG.

Ultrasound examination of thyroid gland was always performed by the same ultrasonographist, in neck hyperextension, on ALOKA SSC-390 ultrasound device with a 7.5MHz ultrasound probe for surface tissues. Lab analyses were performed in the Institute of Nuclear Medicine – Clinical Center Niš (FT4 and TSH by Delfia, 1230 Arcus fluorometer, and anti-TPO and anti-TG by RIA, Clinigamma 1272).

On the basis of the ultrasound results and estimated echogenicity done by a sonographist, the patients were divided into two groups. The group A comprised examinees with estimated normal echogenicity of thyroid gland tissue, while the group B comprised examinees with decreased echogenicity of thyroid gland tissue. The group B was divided into two subgroups, B1 with a mildly decreased echogenicity – lower than the one in the connective tissue, but higher than the neck muscles echogenicity (*m. sternocleidomastoideus*) and B2 – significantly decreased thyroid gland tissue echogenicity – the same or lower echogenicity than the one in neck muscles.

Results was expressed as mean ± standard errors of mean (SEM). Significance of differences between the groups was determined by Student's *t*-test and Mann-Witney *U* test for continuous data. Statistically significant differences were assumed at p less than or equal to 0.05. Degrees of association between continuous variables were evaluated by Spearman's Rank Correlation analysis.

Results

On the basis of the thyroid gland ultrasound screening and estimated echogenicity, the group A comprised 424 (64.6%) patients with normal ultrasound echogenicity. The group B, with the estimated decreased ultrasound echogenicity, comprised 232 (35.4%) patients. The subgroup B 1 comprised 182 (27.7%) patients, and the subgroup B2, with significant hypoecogoenic ultrasound results comprised 50 (7.7%) patients.

In the group A, the increase of anti-TPO value was indicated in 36 (8.5%) examinees while the positive anti-TG were indicated in 44 (10.4%) examinees. In the group B,

with decreased thyroid gland echogenicity, the increase of anti-TPO was indicated in 112 (48.3%), and the positive results of anti-TG in 96 (41.4%) examinees. In the group B1 with decreased thyroid gland echogenicity the increased value of anti-TPO was indicated in 68 (37.3%) examinees, and the positivity of the anti-TG in 56 (30.7%) examinees. In the group B2 with the significantly decreased thyroid gland echogenicity the increase of anti-TPO value was found in 44 (88.0%), and positivity of anti-TG in 40 (80.0%) examinees. There is a significantly higher percentage of examinees with the increase in anti-TPO and anti-TG positivity in the group B, as well as in the subgroups B1 and B2 in relation to the group A ($p < 0.01$ and $p < 0.001$, respectively). There was also statistically significant higher percentage of examinees with the increase in anti-TPO antibodies and anti-TG positivity in the subgroup B2 in relation to the subgroup B1 ($p < 0.001$). The results are shown in Table 1.

There were also the significant differences in mean values of tested parameters: of anti-TPO, FT4 and TSH.

In the group A, the mean value of anti-TPO was 58.5 ± 18.4 U and in the Group B 339.3 ± 57.8 U. The mean value of anti-TPO in the group B1 was 248 ± 45.9 U, and in the group B2 670 ± 116.7 U. Statistically significant higher values of anti-TPO were observed in the groups B, B1 i B2 in relation to the group A as well as statistically significant higher values of the TPO in the group B2 in relation to the group B1 ($p < 0.001$). The mean value of FT4 in the group A was 14.6 ± 5.5 nmol/L and in the group B 10.1 ± 2.9 nmol/L. The mean value of FT4 in the group B1 was 11.2 ± 3.4 nmol/L and in the group B2 8.8 ± 3.8 nmol/L. The statistically significant lower values of FT4 were observed in the groups B, B1 i B2 in relation to the group A as well as the statistically significant lower values of FT4 in the group B2 in relation to the group B1 ($p < 0.001$). The mean value of TSH in the group A was 1.05 ± 0.34 mIU/L, in the group B 5.04 ± 1.98 mIU/L, in the group B1 3.82 ± 2.12 mIU/L and in the group B2 8.75 ± 4.36 mIU/L. The statistically significant higher values of TSH were observed in the groups B, B1 and B2 in relation to the group A, as well as statistically significant higher values of TSH in the group B2 in relation to the group B1, ($p < 0.001$). The results are shown in Table 2.

In relation to TSH values in the group with normal echogenicity, there were 420 (99%) patients with value below 4 mIU/L. There were only 4 patients with values of 4–10 mIU/L, while there was no one with the values over 10 mIU/L. In the group with decreased echogenicity there were 174 (75%) examinees with TSH values below 4 mIU/L, 42 (18.1%) with 4–10 mIU/L and 16 (6.9%) were with TSH values higher than 10 mIU/L. In the group with mildly decreased echogenicity, 162 (75%) patients were with TSH values lower than 4 mIU/L, 16 (8.8%) patients were with TSH values of 4–10 mIU/L, and 4 (2.2%) patients with values higher than 10 mIU/L. In the group with significantly decreased echogenicity 12 (24%) examinees were with TSH values lower than 4 mIU/L, 26 (52%) examinees with 4–10 mIU/L and 12 (24%) were with the values higher than 10 mIU/L. The results are shown in Table 3. Consequently, in the group with normal echogenicity only 4 (1%) examinees were indicated with subclinical hypothyreoidism. In the group with decreased echogenicity the sublinical hypothyreoidism was indicated in 42 examinees while the clinical hypothyreoidism was indicated in 16 examinees. Fifty eight (25%) examinees suffered from thyroid gland altered function. In the group with mildly decreased echogenicity, 16 examinees were indicated with subclinical and 4 with clinical hypothyreoidism.

Table 1

Number of patients in relation to ultrasound echogenicity and anti-TPO and anti-TG presence

Ultrasound echogenicity	Number of patients n (%)	Anti-TPO increased n (%)	Anti-TG positive n (%)
Normal echogenicity (group A)	424 (64.6)	36 (8.5)	44 (10.4)
Decreased echogenicity (group B)	232 (35.4)	112 (48.3**)	96 (41.4**)
Mildly decreased echogenicity (subgroup B1)	182 (27.7)	68 (37.3)*	56 (30.7)*
Significantly decreased echogenicity (subgroup B2)	50 (7.7)	44 (88.0)**†	40 (80.0)** †

***statistically significant difference in relation to the group A ($p < 0.01$); **statistically significant difference in relation to the group A ($p < 0.001$); †statistically significant difference in relation to the group B1 ($p < 0.001$).**
Anti-TPO – thyroid peroxidase antibodies; anti-TG-thyroglobuline antibodies.

Table 2

Mean values of thyroid peroxidase antibodies (anti-TPO), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) in relation to ultrasound echogenicity

Ultrasound echogenicity	Anti-TPO U	FT4 (nmol/L)	TSH (mIU/L)
	mean ± SEM	mean ± SEM	mean ± SEM
Normal echogenicity (group A)	58.5 ± 18.4	14.6 ± 5.5	1.05 ± 0.34
Decreased echogenicity (group B)	339.3 ± 57.8*	10.1 ± 2.9*	5.04 ± 1.98*
Mildly decreased echogenicity (subgroup B1)	248 ± 45.9*	11.2 ± 3.4*	3.82 ± 2.12*
Significantly decreased echogenicity (subgroup B2)	670 ± 116.7*†	8.8 ± 3.8*	8.75 ± 3.36*†

***statistically significant difference in relation to the group A, ($p < 0.001$); †Statistically significant difference in relation to the group B1, ($p < 0.001$).**
SEM – standard error of mean.

Table 3

Number of patients according to the values of thyroid-stimulating hormone (TSH) and ultrasound echogenicity

Ultrasound echogenicity	TSH (< 4mIU/L) n (%)	TSH (4–10 mIU/L) n (%)	TSH > (10 mIU/L) n (%)
Normal echogenicity (group A)	420 (99)	4 (1)	0
Decreased echogenicity (group B)	174 (75)*	42 (181)*	16 (6.9)*
Mildly decreased echogenicity (subgroup B1)	162 (89.0)*	16 (8.8)*	4 (2.2)*
Significantly decreased echogenicity group B2	12 (24.0)*†	26 (52.0)*†	612 (24.0)*†

***Statistically significant difference in relation to the group A, $p < 0.001$; †Statistically significant difference in relation to the group B1, $p < 0.001$.**

Totally 20 (11%) examinees suffered from altered thyroid function. In the group with significantly decreased echogenicity the subclinical hypothyreoidism was found in 26 examinees, while the clinical hypothyreoidism was found in 12 examinees demonstrating that 38 (76%) examinees suffered from altered thyroid function.

There was a moderate negative correlation between anti-TPO and FT4 and high positive correlation between anti-TPO and TSH in the groups with reduced echogenicity. The results are shown in Table 4.

Table 4

Correlation of anti-thyroid peroxidase (TPO) with free thyroxine (FT4) and thyroid-stimulating hormone (TSH)

Hormones	Group A ρ	Group B ρ	Group B1 ρ	Group B2 ρ
FT4	0.09	-0.36	-0.32	-0.35
TSH	0.16	0.58*	0.52*	0.61*

ρ – Spearman's coefficient of correlation; * $p < 0.001$.

Discussion

Ultrasound diagnostics of thyroid gland diseases is generally accepted and indispensable in defining its morphology. In discovering thyroid gland disfunction, we primarily use the presence of characteristic symptoms and determination of thyroid hormone levels and TSH releasing hormone of pituitary gland. Undoubtedly, the majority of disfunctions are followed by the changed morphology, most commonly the increase – struma and/or nodule presence. Modifications of ultrasonographic characteristics of thyroid gland tissue can be present in its altered function. Decrease of ultrasound echogenicity can be one of the signs of altered thyroid function. Our results clearly speak in favor of ultrasound method of thyroid gland examination as an important method when there is a suspicion of its altered function. Decreased tissue echogenicity during the ultrasound screening is usually followed by an increase of thyroid antibodies and the change in its hormone level. In persons with normal echogenicity the presence of TPO and TG antibodies are found only in a minority of examinees, i.e., 8.5% and 10.4%, respectively that is followed by normal values of FT4 and TSH. With decreased echogenicity the percentage of persons with the presence of antibodies is significantly higher – 48.3% with anti-TPO increase and 41.4% with positive anti-TG. The differences were even more significant in the group with significantly decreased echogenicity – 88.0% and 80.0% examinees, respectively. Other authors also discovered that the persons with decreased thyroid gland echogenicity have significantly frequent increase of anti-TPO (30.6%) in relation to the persons with the normal ultrasound results (6.8%), that is, that the percentage of persons with decreased ultrasound echogenicity reaches 45.95% when antibodies are present, and only 12.4% when there is no increase in anti-TPO [7]. The increase in anti-TPO is six times more probable in case there is a decrease of thyroid gland echogenicity. It is shown that there was a significant correlation between the increase of anti-TPO and thyroide disfunction and that even in persons with TSH values of 2–4 mIU/L there is a significantly higher percentage of those with antibodies increase. The prevalence of TSH increase is almost ten times higher, not only in females, but also in males with the anti-TPO increase in relation to those with no increase [6]. Pedersen et al. [1] states that in the group with a decreased echogenicity the percentage of those with chronic autoimmune thyroiditis was 41%, while 30.6% are with increased anti-TPO, and even 46.8% with altered thyroid function [2]. The study that monitored the group of healthy medical workers over three years period found out that all those that evolved thyroid disfunction in this period suffered from decreased ultrasound echogenicity of thyroid gland at the beginning of the monitoring process [3]. The similar results are achieved by Marcocci et al. [8] who stated that all the persons with hypothyreoidism diagnosed within 18 months, were affected by the decreased thyroid gland echogenicity at the beginning of the monitoring process. An early detection of subclinical and clinical hypothyreoidism is important when having in mind that these are the persons with higher risk of atherosclerosis and cardiovascular disease incident as well as obesity [6]. In our study, in the group of examinees with the normal echogenicity we found only 4 (1%) cases of subclinical hypothyreoidism with TSH values of 4–10 mU/L. In the group with decreased echogenicity the percentage was significantly higher and there ware 58 (25%) patients in the whole group affected by sublinical (UZ 42) or clinical hypothyreoidism (UZ 16). Frequency of thyroid gland function decrease was particularly pronounced in the group with significantly decreased echogenicity; even in 76% of patients a disfunction was found in the sense of sublinical or clinical hypothyreoidism. Other authors also found higher frequency of thyroid gland hypoechogenicity in persons with higher TSH values, in relation to the group of per-

sons with normal values [3, 9–13]. It is stated that only 2.2% persons with normal ultrasound echogenicity suffer from subclinical hypothyreoidism. Comparison of various tests for thyroid dysfunction detection showed that the persons with normal echogenicity, normal anti-TPO and with no evidence of chronic autoimmune thyroiditis are 20% less endangered to be affected by thyroid disfunction.

Conclusion

The ultrasound screening of thyroid gland plays and important role in an early detection of thyroid disfunction, i.e., sublinical and clinical hypothyreoidism. Persons with uncharacteristic symptoms and signs and decreased ultrasound echogenicity of thyroid gland require more thorough endocrinological testing and determination of thyroid hormones level, TSH and thyroid antibodies. The testing results imply that the decreased ultrasound echogenicity represents the significant marker of altered thyroid gland function. In these persons we determined the high percentage of subclinical and clinical hypothyreoidism frequency. An early detection of subclinical and clinical hypothyreoidism is significant if keeping in mind that these persons are with higher risk of atherosclerosis and cardiovascular disease incident as well as obesity which emphasises the importance of ultrasound screening in everyday clinical practice.

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Does the blood glucose control have an effect on the success of the painful diabetic neuropathy treatment?

Da li kontrola glukoze u krvi ima efekta na uspeh terapije bolne dijabetesne neuropatije

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Abstract

Background/Aim. Diabetic neuropathy (DN) is the basic complication of diabetes, associated with impared glucoregulation, metabolic distrurbances, microvascular vessel damage and increased cardiovascular risk. We monitored the impact of glucoregulation on the efficacy of painful diabetic neuropathy (PDN) treatment, when all pharmaceutical treatment options were exhausted. **Methods.** Patients (n = 53, both gender, average age 68.3 ± 12.6) with PDN resistant to the pharmacotherapy were treated with the ultrasound-guided local anesthetic (0.5% procaine hydrochloride, 1% lidocaine, 0.25% levobupivacaine) blocks. Neuropathy was confirmed in accordance with the applicable European Federation of Neurological Societies (EFNS) criteria. Glycosylated hemoglobin (HbA1C) and blood glucose levels were monitored before and after therapy and one month after the treatment. Neuropathic pain was confirmed by Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) or *Douleur neuropathique* (DN4) or pain DETECT scales. The pain intensity was assessed by Visual analog scale, Neuropathic pain symptom and Neuropathic pain symptom inventory (VAS, NPS and NPSI, respectively) scales before and after therapy and one month after the treatment. The efficacy of the therapy was assessed as: excellent result (> 50% of pain loss), good result (30%–49% of pain loss and the therapy does not work (< 30% of pain loss). The correlation between glucoregulation and the outcome was examined. **Results.** Because the values of glycenia and HbA1c were not different among patients treated with different local anesthetics, they were presented together. All patients had elevated blood glucose and HbA1C levels before (8.23 ± 2.77 mmol/L and 8.53% ± 2.48% respectively), after (8.43 ± 2.461 mmol/L and 8.85% ± 2.87%, respectively) and one month after the treatment (8.49 ± 2.22 mmol/L and 8.51% ± 2.09%, respectively). The loss of the pain was not result of the decrease in blood glucose and HbA1C blood levels. VAS, NPS, NPSI values were the following before the therapy: 81.53 ± 11.62 mm; 62.00 ± 13.04; 53.40 ± 17.63, respectively; after the therapy: 29.00 ± 9.23 mm; 13.79 ± 6.65; 11.83 ± 7.93, respectively; and one month later: 26.15 ± 8.41 mm; 12.68 ± 6.03; 9.81 ± 7.64, respectively]. There was no correlation between glucoregulation and excellent outcome. **Conclusion.** Even though the disturbance of glucose control is the key factor for the progression of PDN, it is not significant for the outcome of the pain treatment. New investigations are required.

Key words:
diabetic neuropathies; blood glucose; blood chemical analysis; surveys and questionnaires; anesthetics, local; nerve block; pain measurement; treatment outcome.

Apstrakt

Uvod/Cilj. Dijabetesna neuropatija (DN) je osnovna komplikacija dijabetesa, udružena sa poremećajem glikoregulacije i metabolizma, oštećenjem malih krvnih sudova i povišenim kardiovaskularnim rizikom. U istraživanju je praćen uticaj glikoregulacije na efikasnost lečenja bolne dijabetesne neuropatije (BMDN) rezistentne na medikamentno minimalno invazivnom terapijom. **Metode.** Kod bolesnika (n = 53, oba pola, starosti 68,3 ± 12,6) sa BMDN primenjena je minimalno invazivna terapija – lokalnim anesteticima (0.5% prokain hidrohlorid, 1% lidokain, 0.25% levobupivakain) ultrazvučno vođenim blokovima. Neuropatija je potvrđena u skladu sa važećim kriterijumima Evropske federacije neurološkog udruženja (EFNU). Glikoregulacija je preko vrednosti glikemije i glikozilirani hemoglobina (HbA1c), pre lečenja,

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nakon ciklusa terapije i posle jednog meseca od završetka terapije. Neuropatski bol potvrđen je skalama *Leeds assessment of neuropathic symptoms and signs* (LANSS) ili *Dopleur neuropathique* (DN4) ili *pain DETECT* skale za utvrđivanje bola. Intenzitet bola je ocenjeni su vizuelnom analognom skalom, neuropatskom skalom simptoma bola i listom simptoma neuropatskog bola. Primenjena je perineuralna blokada lokalnim anesteticima, pod kontrolom ultrazvuka. Efikasnost terapije određivana je procentom smanjenja bola: > 50% – odličan rezultat, 30%–49% – dobar rezultat i < 30% – terapija ne deluje. Ispitana je korelacija glikoregulacije (glikemije i nivoa HbA1c) i ishoda lečenja. **Rezultati.** Svi bolesnici su imali povišenu vrednost glikemije i HbA1C na početku lečenja (8,23 ± 2,77 mmol/L i 8,53% ± 2,48%, redom), na kraju terapije (8,43 ± 2,46 mmol/L i 8,85% ± 2,87%, redom) i posle meseca praćenja (8,49 ± 2,22 mmol/L i 8,51% ± 2,09%, redom). Prestanak bola nije bio u vezi sa smanjenjem glikemije [skale za procenu bola redom pre terapije: 81,53 ± 11,62 mm; 62 ± 13,04; 53,40 ± 17,63; jedan mesec posle terapije: 29 ± 9,23 mm; 13,79 ± 6,65; 11,83 ± 7,93; i jedan mesec kasnije; 26,15 ± 8,41 mm; 12,68 ± 6,03; 9,81 ± 7,64]. Nije bilo korelacije između poremećaja glikoregulacije i odličnog terapijskog odgovora.

Ključne reči:
dijabetesne neuropatije; glikemija; krv, hemijske analize; upitnici; anestetici, lokalni; blokada živca; bol, merenje; lečenje, ishod.

Introduction

Diabetic neuropathy (DN) is the basic complication of diabetes that was first described by Dyck et al. in 1880 as symmetrical sensorimotor polyneuropathy. For the first time, it was associated with impared glucoregulation, metabolic disturbances, microvessels damage and increased cardiovascular risks [1, 2]. Hyperglycemia is the essential disorder in the pathogenesis of the DN development in both types of diabetes [2–15]. It increases the polyol pathway activity [1, 4, 6, 16]: glucose is transformed into sorbitol, catalyzed by aldose reductase, with the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$ [4, 6]. Sorbitol oxidizes to fructose with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. Long-lasting hyperglycemia elevates the affinity of aldose reductase for glucose. Inhibitors of aldose reductase activity are very effective in preventing the DN development in animal model, but clinical application is limited by a dose-dependent toxicity [16]. The sorbitol is accumulated in a cell and leads a cell in to the osmotic stress because it cannot pass through the cell membrane, but it does not cause the damage to neurons [17–19]. Toxicity of hyperactive polyol pathway is in the increased turnover of NADPH to NAD^+, in the decreased reduction and regeneration of glutathione, in the increase of advanced glycation end products (AGEs), and in the activation of protein kinase C (PKC) isoforms [17–19]. The intracellular decrease of NADPH level leads to the depletion of nitric oxide formation and blood supply to the nerves because the nitric oxide is a very strong vasodilatator [4, 6, 20]. All this causes the nerve damage and leads to the progressive and ascendant development of the distal-to-proximal diabetic neuropathy in an extremity [21].

Cellular glutathione depletion increases toxic products [17] and the level of oxidative stress [1, 4, 6] what is another basic biochemical mechanism for the DN development confirmed in diabetic animal models [22]. Intracellular hyperglycemia causes the autoxidation of glucose and its metabolites, an increased formation and expression of receptors for AGEs and its activating ligands. The accumulation of AGEs products is associated with the activation and proliferation of microglia and astrocytes-morphological changes in the central nervous system, six years after the presence of continuous neuropathic pain [1–6]. Intracellular hyperglycemia damages the mitochondrial function and leads to over-activity of the hexosamine pathway [23–25]: reactive nitrogen species and the peroxynitrite in particular are very toxic [26–29]. The results of clinical application of antioxidants are contradictory as well: alpha-lipoic acid can have light beneficial therapeutic effect [30, 31] or ensure positive prospects for the improvement [32, 33].

Diabetic neuropathy is commonly manifested as the loss of sensitivity with the chronic neuropathic pain: pain lasting for more than three months accompanied by allodynia and hyperpathia [4, 6]. The development of chronic neuropathic pain is also explained by disturbances of action potentials [4, 6, 34, 35]. The central nervous system (CNS) interprets it as a pain (allodynia and hyperpathia) [4, 7, 36]. The up-regulation of voltage-depended Na-canals (Nav) is confirmed in neuropathic pain models [37]. The Nav accumulates at the damaged sites of axons what leads to ectopic electrical discharges and the Nav hyperexcitability and the increased bursts of electrical impulses in the nociceptive system at the dorsal corn of the spinal cord [38]. Such bursts damage the antinociceptive gate-control mechanism and the P substance expression [38]. Disturbances in the Nav expression, structure and function cause the neuronal hyperexcitability or the development of neuropathic pain [39]. The CNS hyperexcitability and the Nav involvement in the development of this process equalize the pathophysiology of chronic pain with epilepsy what is confirmed by therapeutically used antiepileptic drugs [40].

Local anesthetic agents (LA) block the Nav canals [41]. Over the last seven to ten years, the LA application has been introduced into the chronic neuropathic pain therapy [42–45]: locally as a plaster gel or injection-solution, it is applied into the area near the damaged nerve structure [44]. The minimally invasive application of the injection (block) into the area around the nerve structure is extremely safe if done in a real-time and was ultrasound-guided. The minimal LA dose is used because it is applied into the area immediately surrounding the damaged structure. The LA application on a daily basis blocks the changed Nav, and interrupts the increased bursts of electrical impulses passing down to the dorsal corn of the spinal cord [45, 46].

Strong glycemia control reduces the development and progression of diabetic neuropathy up to 64% [1–6]. Therefore,

it is recommended to control the blood glucose level four times a day, and the blood HbA1C level once a month [4, 6]. Considerably less important risk factors for the DN development are hyperlipidemia, hypertension, smoking, alcohol abuse, obesity, age, and the duration of diabetes [1–6].

Does the poor glucose control have the impact on the DN therapy outcome? Can the chronic pain be relieved by the suppression of basic pathophysiological mechanisms involved in chronic neuropathic pain, regardless of the glucose control? It is about the pain that disturbs all aspects of life, not only of the patient but also of his/her family members. That is the main issue this study deals with.

Methods

Patient selection

This study included 53 adult patients of both genders (24.5% males and 75.5% females), average age 68.4 ± 12.6 years with chronic painful diabetic neuropathy in the lower extremities. The duration of the pain was longer than three months and less than six years (3.2 ± 1.78 years). All patients had poor glycemic control (it was measured four times a day) and elevated HgA1C values. The 84.9% of the patients were smokers with mildly elevated values of arterial pressure (96.2%) contolled using only one type of medicine given at a low dose. None of the patients abused alcohol. Medical therapy benefits were exhausted: ineffective (the pain measured by VAS scale was > 30 mm) or side-effects were intolerable, and the therapy was discontinued (at the patient's request or the physician's judgment that vital functions or normal daily activities of the patient are seriously threatened). The neuropathic pain in lower extremities was confirmed by the LANSS (LANSS ≥ 12 points), or pain DETECT scale (≥ 19 points) or DN4 scale (≥ 4 points). All the scales were used for each patient. The diabetic neuropathic pain was confirmed in accordance with the EFNS recommendations [47]: clinical and neurological examination, the elecromyoneurographic examination of lower extremities. All patients were mentally healthy and intelectually capable of understanding their participation in the study and gave their informed consent for it. The exclusion criteria were: ischaemical cerebral and/or myocardial diseases; metabolic mitochondrial diseases; liver diseases; respiratory or metabolic acidosis; arrhythmias; hemorrhagic diathesis; psychiatric illnesses; epilepsy; CNS diseases confirmed by magnetic resonance imaging (MRI); three or more evidence-based risk factors for stroke or acute myocardial infarction; evidence-based allergic reaction to local anasthetics; unregulated arterial hypertension.

The inclusion criteria were normal values of the following biochemical analyses: complete blood count, sedimentation rate, serum proteins, B12 and D3 vitamin blood level, the blood level of C3 and C4 components of the complement, the blood tumor marker values [β2 microglobulin, carcinoembryonic antigen – CEA, alpha-fetoprotein (α FP), cytokeratin fragment 21 (CYFRA 21), neuron specific enolase (NSE), carbohydrate antigens (CA) 72.4, CA 125, CA 15.3, CA 19.9; for male: prostate specific antigen (PSA), free PSA (fPSA), international normalized ratio (INR) and activated partial thromboplastin time (APTT) values, hepatic ensimes [aspartate aminotransferase (AST); alanine aminotrasferase (ALT); gamma-glutamil transferase (GGT); lactate dehydrogenase (LDH), blood levels of the urea, creatinine, uremic acid, the urine levels of amylase, triglycerides, high-density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol blood levels. The urine value of the ketones was allways less than two pluses (at the start and the end of the therapy, and one month after the therapy). Based on the examination of the blood vessels of lower extremities by Doppler sonography and multisliced computed computed tomography (MSCT) angiography, more than 30% stenosis were excluded.

The glycemic values were measured four times a day (the phosphorylation of glucose by the hexokinase method on a Siemens Dade Dimension RxL Max chemistry analyzer), on the basis of which the mean blood glucose level was calculated before the introduction of the therapy, at the end of the therapy, and one moth after the treatment. The HbA1C blood level was also measured [by the turbidimetric inhibition immunoassay (TINIA), SIEMENS Dimension RXLMAX analyzer] before the therapy, at the end of the therapy and one month after the completion of the treatment.

The ultrasound-guided treatment (in B-mod and color doppler mod; on the Toschiba Aplio 5000 Ultrasound Maschine with linear probe 7–18 MHz, the programe for periferal nerves and muscles) was performed using injections – blocks with local anesthetics (0.5% procaine hydrochloride, 1% lidocaine, 0.25% levobupivacaine), under sterile conditions. The blocks were given five days a week until the pain was lost [48] and two blocks more until the positive therapeutic effects were observed, but no more than ten blocks.

The blocks were administered into the lower extremities: „three in one“ blocks – lower (caudal) lumbar plexus block (always 3 mL of LA only) and subgluteal sciatic nerve blocks (always 5 mL of LA only). Prior to the initiation of the treatment, when the procedure was explained to the patients and informed consents were obtained from them, the pain was assessed by the VAS, NPS, NPSI, and pain DETECT Scale. In the same way, the pain was assessed after the treatment and one month upon the completion of the therapy.

The outcome of the chronic neuropathic pain treatment was assessed by listed scales and numerical values were interpreted as follows: excellent results (the pain intensity is reduced by ≥ 50% as compared to the initial pain evaluation); good results (the pain intensity is reduced by 30%–49% when compared to the initial pain evaluation) and unsatisfactory (the pain intensity is reduced by < 30% as compared to the initial pain evaluation) [47]. After that, the correlation with the initial glycemia and HbA1C values was analyzed.

Ethics

All the research procedures were approved by the Military Medical Academy Ethical Committee, Belgrade, Serbia (Ethical Committee Meeting – 30th November 2015.).

Statistical analysis

All the data were collected and processed using the SPSS program for Windows. They are presented in the standard way as the mean values and the standard deviation. Regression and correlation analyses between parameters for the blood glucose levels (glycemia and HbA1C values) and the treatment results (VAS, NPS, NPSI and the pain DETECT scale values) were carried out.

Results

Because the values of glycenia and HbA1c were not different among patients treated with different local anesthetics, they were presented together.

Glycemia and HbA1C values and numerical values of the VAS, NPS, NPSI and pain DETECT scales before and after the therapy, and one month after the treatment are presented in Table 1.

The mean value of the VAS scale was 29 ± 9.232 after the therapy and 26.15 ± 8.413 one month after the treatment - excellent outcome (more than 50% of pain disappeared).

Table 2 presents the correlation between glycemia and HbA1C value and the numerical values on the pain scales (pain was measured by VAS, NPS, NPSI, and pain DETECT scale). The intensity of pain was measured after therapy and one month after therapy finished.

There was no correlation between glycemia as well as HbA1C values and numerical values of the pain scales (p > 0.5 Friedman's ANOVA).

Table 1

The values of the glycemia, HbA1c and pain scales

Parameters	Before therapy	After therapy	1 month after the treatment
Glycemia (mmol/L), mean ± SD	8.23 ± 2.771	8.43 ± 2.461	8.49 ± 2.224
HbA1c (%), mean ± SD	8.53 ± 2.478	8.85 ± 2.872	8.51 ± 2.090
VAS (mm), mean ± SD	81.53 ± 11.62	29 ± 9.232	26.15 ± 8.413
NPS (points), mean ± SD	62 ± 13.041	13.79 ± 6.649	12.68 ± 6.025
NPSI (points), mean ± SD	53.4 ± 17.637	11.83 ± 7.932	9.81 ± 7.636
pain DETECT (points), mean ± SD	25.58 ± 5.891	7.87 ± 3.883	7.53 ± 3.662

HbA1c – glycosylated haemoglobin; VAS – Visual analogue scale; NPS – Neuropathic pain scale; NPSI – Neuropathic pain symptom inventory; SD – standard deviation.

Table 2

Correlation between glycemia and glycosylated haemoglobin (HbA1c) and numerical values on the pain scales

Scales	Glycemia		HbA1c		p
	Correlation coefficient	p	Correlation coefficient		
VASpp	-0.05	0.698	-0.004	0.978	
VASm	-0.127	0.366	0.062	0.659	
NPSpp	-0.033	0.816	0.076	0.594	
NPSIpp	-0.111	0.431	-0.110	0.431	
NPSIm	-0.089	0.524	-.116	0.403	
pDETpp	-0.081	0.562	0.179	0.199	
pDETm	0.023	0.868	0.158	0.257	

Pp – pain after therapy; m – pain one month after therapy; pDET – pain DETECT;
For other abbreviations see under Table 1.

Discussion

In the course of the investigation, a group of mostly older (one of the additional risk factor for the DN development) and female patients [1–6] was formed. The gender changes the pain experience due to differences in psychosocial mechanisms, the hormonal status and activity, the function of the opioid system and the NMDA receptors, all of which required different approaches to the pain therapy in men and women [49].

The main way to control glycemia in diabetic patients was the measurement of blood glucose and HbA1C levels. To prevent the DN development, the recommended value for HbA1C that should have been maintained was < 7%, while the glycemia level should have been within the range of 0–13 mmol/L or, if it is measured two hours after a meal, it was desirable to be < 18 mmol/L [1–6]. It was also recommended to measure glycemia four times a day [4].

We formed the group of subjects with metabolic disbalance and poor glucoregulation. Poor glucoregulation leads to the diabetic neuropathy in both types of diabetes, and, the majority of patients in our investigation are with type 2 diabetes (50 out of 53 patients).

In addition to the bad glycemia control, smoking was the factor that contributed to the DN development in all the subjects of our study group, while the hypertension was regulated with minimal dose of only one type of drug. Mild hypertension was recommended for the prevention of hyperglycemic tissue damage, better tissue perfusion, particularly of the CNS tissue, but the values should not exceed 130/80 mmHg [1, 4, 6].

Other metabolic and vascular neuropathies identified using Doppler sonography or MSCT angiography of lower extremities and on the basis of laboratory results were excluded.

There was no correlation between glycoregulation (the values of blood glucose and HbA1c levels) and excellent therapy results with the LA according to the pain scales values.

The LA use in the chronic pain treatment is actual again, after ultrasound and Doppler applying for nerve and vascular structure real-time visualization [50]. Local application was safe and easy, required minimal LA doses and avoided the gastrointestinal tract. Administration of a single-shot LA dose on a daily basis resulted in the loss of the pain within few months, frequently without the need to introduce any other type of medication for the neuropathic pain. The Food and Drug Administration (FDA) recommendation is that local anesthetics should be only used for the postherpetic neuralgia pain treatment [51]. The ultrasound-guided block allows local anesthetics to spread along the nerve proximally and distally, which was confirmed by the MRI images taken immediately after blocks were given [52].

Conclusion

The therapy of the medicine-resistant pain in the diabetic neuropathy by the Nav canals block with local anesthetics proved to be effective in our investigation, regardless of the increased blood glucose and HbA1C levels. It may lead to the assumption that they are the only mechanisms involved in the DN development while other mechanisms are responsible for the maintenance of the chronic pain. New investigations are required to provide answers to many pathophysiological enigmas in this chronic pain.

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Quality of life in patients early after surgery

Kvalitet života bolesnika u ranom postoperativnom toku

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Abstract

Background/Aim. Quality of life in patients early after elective surgery is related to postoperative pain and recovery rate. The aim of this study was to compare immediate preoperative and early postoperative quality of life after three common elective surgical interventions in hospital settings. **Methods.** Population of this prospective cohort study included patients who underwent one of the three surgical interventions: elective laparoscopic cholecystectomy (n = 40), open inguinal hernia repair (n = 40) or excision of pilonidal sinus (n = 40). Primary outcome of the study was quality of life measured once-daily, starting from the day before surgery, and then each postoperative day. It was measured by visual analogue scale (VAS) and by Serbian translation of short questionnaire on quality of life developed by World Health Organization. **Results.** Postoperative quality of life dropped to the lowest level on the first postoperative day, regardless of the type of surgery. The drop was the most pronounced in physical and psychological aspects of quality of life (e.g. after cholecystectomy from 15.4 ± 2.5 to 12.5 ± 2.0, and from 15.9 ± 2.0 to 14.9 ± 2.1, respectively) while social and environmental aspects were the least affected by the surgery (e.g., after excision of pilonidal sinus from 16.3 ± 2.6 to 15.7 ± 2.1, and from 14.3 ± 2.6 to 14.1 ± 2.2, respectively). Quality of life was rapidly restored on the second postoperative day, and on the last day before discharge of the patient from hospital it surpasses preoperative level (e.g., after open inguinal hernia repair from 14.6 ± 3.6 to 15.2 ± 3.0. **Conclusions.** Minor elective surgical interventions are associated with only moderate (less than 25%) and short (one day) immediate postoperative decrease in quality of life, which is followed by increase on discharge from hospital to the levels, higher than preoperative one.

Key words:
surgical procedures, operative; quality of life; pain, postoperative; postoperative period; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Kvalitet života bolesnika u ranom postoperativnom toku posle elektivnih operacija povezan je sa postoperativnim bolom i brzinom oporavka. Cilj ove studije bio je da uporedi kvalitet života bolesnika u neposrednom postoperativnom periodu posle tri česte elektivne hirurške intevencije u bolničkim uslovima. **Metod.** Populaciju ove prospektivne kohortne studije činili su bolesnici podvrgnuti jednoj od sledećih hirurških intervencija: elektivna laparoskopska holecistektomija (n = 40), otvorena operacija preponske kile (n = 40) ili ekscizija pilonidalnog sinusa (n = 40). Primarni ishod studije bio je kvalitet života meren svakodnevno, počev od dana koji prethodi operaciji, a zatim svakog postoperativnog dana. Kvalitet života bio je meren vizuelnom analognom skalom (VAS) i prevodom na srpski Kratke forme upitnika za kvalitet života Svetske zdravstvene organizacije. **Rezultati.** Postoperativni kvalitet života opao je na najniži nivo prvog postoperativnog dana, bez obzira na vrstu hirurške intervencije. Pad je bio najizraženiji u fizičkom i psihološkom domenu upitnika (npr. posle holecistektomije sa 15,4 ± 2,5 na 12,5 ± 2,0 i sa 15,9 ± 2,0 na 14,9 ± 2,1, po redosledu), dok su socijalni i domen okruženja bili najmanje pogođeni operacijom (npr. posle ekscizije pilonidalnog sinusa sa 16,3 ± 2,6 na 15,7 ± 2,1, i sa 14,3 ± 2,6 na 14,1 ± 2,2, po redosledu). Kvalitet života se brzo vratio na početni nivo drugog postoperativnog dana, da bi poslednjeg dana pred otpust iz bolnice postigao viši nivo od preoperativnog (npr. posle otvorene operacije preponske kile sa 14,6 ± 3,6 na 15,2 ± 3,0). **Zaključak.** Manje elektivne hirurške intervencije su praćene umerenim (ispod 25%) i kratkim (jedan dan) neposrednim postoperativnim smanjenjem kvaliteta života, za kojim sledi porast sve do nivoa višeg od preoperativnog, na otpustu iz bolnice.

Ključne reči:
hirurgija, operativne procedure; kvalitet života; bol, postoperativni; postoperativni period; upitnici.

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Introduction

Quality of life is one of the most important outcomes of surgery which is increasingly used for comparing efficacy and safety of alternative treatment strategies. Quality of life has five key dimensions which reflect capability of patients to conduct certain functions: moving, self-care, performing usual activities, presence of pain and other complaints, and presence of anxiety or depression [1]. Although there are several generic instruments which could be used for measuring quality of life after surgery, like Short Form Health Survey (SF-36), Euro QoL Five Dimensions Questionnaire (EQ 5D) and the World Health Organization Quality of Life (WHO-QOL-BREF) [2–4], the last one is more comprehensive than EQ 5D and could be used without cost (unlike SF-36). Reliability and validity of the WHOQOL-BREF have been recently tested in Serbian population [5].

Quality of life in patients early after surgery is related to postoperative complications, pain and recovery rate [6]. Several other factors were also shown to be associated with quality of life early after surgery such as: type of surgery, type of anesthesia, age and sex of the patients, mechanical ventilation, duration of surgery, co-morbidities, methods of postoperative pain management, etc. [7–9].

Although it was shown that quality of life early after surgery gradually increases throughout the postoperative period, rate of increase is still unknown for majority of surgical techniques [10, 11]. Besides, relation of immediate preoperative and early postoperative quality of life is also unknown, as well as the factors associated with [12].

The aim of this study was to compare immediate preoperative and early postoperative quality of life after three common elective surgical interventions in hospital settings: laparoscopic cholecystectomy, open inguinal hernia repair and excision of pilonidal sinus.

Methods

The study design

The study design was of prospective cohort type, with three parallel cohorts: the patients undergoing elective laparoscopic cholecystectomy, open inguinal hernia repair or excision of pilonidal sinus. It was conducted at the single center (Surgery Clinic, Military Medical Academy, Belgrade, Serbia) from January to June 2016. The study obtained approval prior to commencement from the Ethics Committee of Military Medical Academy, Belgrade.

Population and the sample

The study population included patients who were admitted to Surgery Clinic and then underwent one of the three surgical interventions during the study period, if the following inclusion criteria were met: age over 18 years, signed informed consent for participation in the study, elective nature of surgery and full consciousness and accountability throughout the study. The exclusion criteria were: refusal to sign informed consent, pregnancy, history of major mental diseases, dementia, mental retardation and pre- or postoperative delirium. The study sample was consecutive and the cohorts were locked once the pre-defined number of participants was reached.

The study outcome and variables

Primary outcome of the study was quality of life measured once-daily, starting from the day before surgery, and then each postoperative day. Quality of life was measured by visual analogue scale (VAS) and by Serbian translation of short form of quality of life questionnaire (with 26 questions) developed by World Health Organization (WHOQOL-BREF). The questionnaire has 4 domains (physical health, psychological, social relationships and environment) and the first two questions are integrated in general estimate of quality of life. The responses are transformed to the scale with minimum (zero) and maximum (20 points) quality of life. Previously validated Serbian translation of the questionnaire was obtained from the World Health Organization with permission to use it in this study. The questionnaire was administered by the investigators. The VAS scale was presented to the patients as a line 100 mm long drawn on the paper, marked at the beginning with 0 and at the end with 100 and patients were asked to show with a pencil the point on the line which reflected their overall quality of life at that moment. Secondary outcomes of the study were duration of hospitalization and rate of postoperative complications.

The following independent and potentially confounding variables were collected from the patient files: age, sex, level of education, marital status, diagnosis, type of surgical intervention, duration of surgery, type of anesthesia (general inhalational or local infiltrative), utilization of postoperative analgesia [either ketorolac (i.m., i.v.), diclofenac (i.m., i.v.) or paracetamol (i.v.)], postoperative headache and postoperative day when a patient started oral intake.

Sample size calculation

Sample size was calculated using G-Power software, version 3.1 [13]. In the study of Acar et al. [14] significant difference was noted in quality of life of patients operated on with two different surgical techniques (58.78 ± 15.85% in one and 70.31 ± 19.38% in another group). Based on the effect size calculated from the study [14], and using two-sided Student's *t*-test, with expected power of the study of 80% and probability of type I error of 0.05, minimal size of each of the study cohorts was 38 patients.

Statistics

Descriptive statistics of the study data included measures of central tendency (mean and median), measures of variability (standard deviation) and percentages. Normality of data distribution for each of the variables was tested by Shapiro-Wilk's test. When normality was confirmed, differences among the cohorts were tested by one-way ANOVA,

and for the opposite nonparametric Kruskal-Wallis analysis of variance was used. Also, when normality of the data distribution was confirmed, differences in the same variable among the days of follow-up were tested by one-way repeated measures ANOVA, and for the opposite Friedman's test was used. Tamhane *post-hoc* test was used to make pairwise comparison among the groups with nomal distrubution of data, and for the opposite Wilcoxon matched pair sign test was used. A multiple linear regressions were calculated for each cohort to predict general estimate of quality of life on the last day of follow-up (before the patients were discharged from hospital). The results were considered significant if probability of null hypothesis was less than 0.05. All calculations were made by the SPSS software, version 18.

Results

The three study cohorts included 40 patients each. Characteristics of the patients within the study cohorts are shown in the Table 1.

The quality of life in all three cohorts, as measured by VAS, general estimate and by the four domains dropped on the first postoperative day relative to preoperative estimate, and then increased above the preoperative level on postoperative days two and three (Table 2). While the quality of life preoperatively was the highest in patients awaiting inguinal hernia repair, it reached the highest values in patients who underwent excision of pilonidal sinus postoperatively (Table 2).

A multiple linear regressions were calculated for each cohort to predict general estimate of quality of life on the last day of follow-up (before the patients were discharged from hospital) by the first two questions of WHOQOL-BREF, based on sex, age, duration of surgery, postoperative headache and onset of oral intake after the surgery. The regression for cholecystectomy cohort was not significant ($F = 1.274$; $p = 0.301$) and explained only 14.1% of variability ($R^2 = 0.141$) in quality of life based on the following predictors: sex ($B = -0.048$; $p = 0.953$) age ($B = -0.043$; $p = 0.092$), duration of surgery ($B = -0.060$; $p = 0.194$) and postoperative headache ($B = 1.119$; $p = 0.188$). The regression for hernia repair cohort was not significant ($F = 1.979$; $p = 0.120$) and explained 30.1% of variability ($R^2 = 0.301$) in quality of life based on the following predictors: sex ($B = -0.576$; $p = 0.674$) age ($B = -0.104$; $p = 0.071$), duration of surgery ($B = 0.136$; $p = 0.152$), postoperative headache ($B = 1.083$; $p = 0.538$) and onset of oral intake after the surgery ($B = -2.333$; $p = 0.279$). Finally, the regression for pilonidal sinus cohort was not significant ($F = 0.852$; $p = 0.508$) and explained 14.0% of variability ($R^2 = 0.140$) in quality of life based on the following predictors: sex ($B = 0.688$; $p = 0.572$) age ($B = 0.130$; $p = 0.173$), duration of surgery ($B = 0.056$; $p = 0.411$) and postoperative headache ($B = -1.027$; $p = 0.366$).

Discussion

Our study showed that postoperative quality of life drops to the lowest level on the first postoperative day, regardless of the type of surgery. The drop is the most pronounced in physical and psychological aspects of quality of life, while social and environmental aspects are the least affected by the surgery. Although statistically significant, the decrease of quality of life on the first postoperative day is moderate, and never overcomes 25% of the preoperative level. Quality of life also is rapidly restored on the second postoperative day, and on the last day before discharge of the patient from hospital it surpasses preoperative level. No predictors of postoperative quality of life were found in our study.

Table 1

Characteristics of the patients within the study cohorts

Characteristics	Laparoscopic cholecystectomy (n = 40)	Open inguinal hernia repair (n = 40)	Excision of pilonidal sinus (n = 40)
Sex: male/female, n	18/22	30/10	31/9
Age (years), mean ± SD	55.3 ± 16.1	56.7 ± 14.1	27.3 ± 6.9
Duration of surgery (minutes), mean ± SD	62.5 ± 8.2	52.2 ± 6.1	34.8 ± 7.1
Education level, n elementary/high school/higher education	11/16/13	12/12/16	5/17/18
Marital status, n married/unmarried	31/9	27/13	12/28
Type of anesthesia, n general inhalatory/local infiltrative	40/0	40/0	0/40
Use of postoperative analgesia, n yes/no	40/0	40/0	40/0
Complications of surgery, n yes/no	0/40	1/39	3/37
Length of hospitalization, n 2 days/3 days/4 days/5 or more days	4/25/10/1	0/11/18/11	14/23/1/2
Postoperative headache, n yes/no	21/19	26/14	19/21
Onset of oral intake, n 1st day/2nd day	0/40	7/33	5/35

n – number of patients; SD – standard deviaton.

Table 2

Quality of life estimates by day and surgical intervention (mean ± standard deviation)

Type of intervention and outcome measure	Preoperative day	First postoperative day	Second postoperative day	Third postoperative day	Significance of difference
Laparoscopic holecystectomy (n = 40)					
VAS (0 – 100)	63.0 ± 17.3	51.5 ± 19.2	63.6 ± 14.8	69.1 ± 18.1	p = 0.000* $Wilcoxon_{p,1}$: p = 0.002 $Wilcoxon_{1,2}$: p = 0.000 $Wilcoxon_{1,3}$: p = 0.003 $Wilcoxon_{2,3}$: p = 0.034
General estimate (0 – 20)	14.1 ± 2.3	12.6 ± 2.9	13.8 ± 2.3	13.6 ± 3.1	p = 0.001* $Wilcoxon_{p,1}$: p = 0.005 $Wilcoxon_{1,2}$: p = 0.002
Physical health (0 – 20)	15.4 ± 2.5	12.5 ± 2.0	14.4 ± 1.9	14.1 ± 2.3	p = 0.003* $Wilcoxon_{p,1}$: p = 0.000 $Wilcoxon_{p,2}$: p = 0.010 $Wilcoxon_{1,2}$: p = 0.000 $Wilcoxon_{1,3}$: p = 0.038
Psychological (0 – 20)	15.9 ± 2.0	14.9 ± 2.1	15.7 ± 1.8	15.2 ± 2.3	p = 0.018* $Wilcoxon_{p,1}$: p = 0.003 $Wilcoxon_{1,2}$: p = 0.006
Social relationships (0 – 20)	15.4 ± 2.2	14.9 ± 2.3	14.9 ± 2.3	14.2 ± 1.9	p = 0.197
Environmental (0 – 20)	14.4 ± 1.9	13.8 ± 1.7	14.3 ± 1.4	13.7 ± 1.6	p = 0.001* $Wilcoxon_{p,1}$: p = 0.011 $Wilcoxon_{p,3}$: p = 0.035 $Wilcoxon_{1,2}$: p = 0.005
Excision of pilonidal sinus (n = 40)					
VAS (0 – 100)	61.8 ± 24.7	62.3 ± 21.8	78.1 ± 17.4	-	p = 0.007* $Tamhane_{p,2}$: p = 0.006 $Tamhane_{1,2}$: p = 0.006
General estimate (0 – 20)	13.9 ± 3.7	13.6 ± 3.0	15.8 ± 2.5	-	p = 0.018* $Tamhane_{p,2}$: p = 0.047 $Tamhane_{1,2}$: p = 0.005
Physical health (0 – 20)	15.2 ± 3.2	13.5 ± 2.3	14.9 ± 2.0	-	p = 0.029* $Tamhane_{1,2}$: p = 0.047
Psychological (0 – 20)	16.7 ± 2.0	16.5 ± 2.0	17.3 ± 2.0	-	p = 0.260
Social relationships (0 – 20)	16.3 ± 2.6	15.7 ± 2.1	16.5 ± 2.3	-	p = 0.472
Environmental (0 – 20)	14.3 ± 2.6	14.1 ± 2.2	15.4 ± 2.0	-	p = 0.078
Open inguinal hernia repair (n = 40)					
VAS (0 – 100)	70.5 ± 20.0	53.1 ± 20.8	65.9 ± 20.2	73.3 ± 22.0	p = 0.000* $Tamhane_{p,1}$: p = 0.002 $Tamhane_{1,2}$: p = 0.043 $Tamhane_{1,3}$: p = 0.002
General estimate (0 – 20)	14.6 ± 3.6	12.4 ± 2.6	14.0 ± 2.7	15.2 ± 3.0	p = 0.000* $Wilcoxon_{p,1}$: p = 0.001 $Wilcoxon_{1,2}$: p = 0.000 $Wilcoxon_{1,3}$: p = 0.000 $Wilcoxon_{2,3}$: p = 0.001
Physical health (0 – 20)	16.1 ± 2.8	13.5 ± 2.5	14.8 ± 2.1	15.4 ± 2.8	p = 0.000* $Tamhane_{p,1}$: p = 0.000 $Tamhane_{1,3}$: p = 0.039
Psychological (0 – 20)	16.5 ± 2.3	15.4 ± 2.1	16.1 ± 2.5	17.1 ± 2.5	p = 0.020* $Tamhane_{1,3}$: p = 0.020
Social relationships (0 – 20)	15.9 ± 2.5	14.5 ± 2.1	14.8 ± 2.1	15.7 ± 2.1	p = 0.019* $Tamhane_{p,1}$: p = 0.070
Environmental (0 – 20)	14.6 ± 2.1	13.6 ± 2.2	13.8 ± 2.0	14.3 ± 2.2	p = 0.139

*** significant difference among the days of measurement; VAS – Visual Analogue Scale.**

Since postoperative pain is one of the most important determinants of early postoperative quality of life, it is not surprising that we found sudden drop of quality of life on the first postoperative day, and then restoration on as early as the next postoperative day. In the study of Saadati et al. [15] postoperative pain after laparoscopic cholecystectomy was about 2.5 (on VAS scale from 0 to 10) on the first postoperative day, only to drop to 1.7–1.3 on the second postoperative day and to less than 0.2 on the third postoperative day. Ciftci et al. [16] showed similar experience with open inguinal hernia repair: the patients reported significant pain on the first postoperative day (about 5.7 on VAS scale from 0 to 10), only to leave the hospital on the second day without significant complaints on pain. Patients who underwent excision of pilonidal sinus in the study of Tavassoli et al. [17] scored postoperative pain in the first day 4.7 ± 1.6, and only 1.9 ± 1.2 on the fourth postoperative day.

With minor elective operations which do not alter physiological functions of the patients significantly, postoperative pain is the most pronounced on the first postoperative day and then rapidly dissipates in the next few days; quality of life tightly follows these changes, as we noted in our study.

Only moderate drop in quality of life on the first postoperative day and rapid restoration on the second one observed in our study could also be explained by early gain of mobility, independence of medical aid and early return to everyday activities achieved with laparoscopic cholecystectomy and other two investigated operations. In the study of Lezana-Perez et al. [18], the patients were discharged from hospital as early as on average 1.49 days after laparoscopic cholecystectomy returning immediately to their normal life activities.

It is not surprising either that quality of life on the day of discharge from hospital is higher than preoperatively. The patients are cured with these elective operations, and old complaints are no longer present, as given in the study of Pierides et al. [19], who showed increase in quality of life after open inguinal hernia repair in elderly patients. Similar was demonstrated in the study of Ertan et al. [20] with quality of life in patients after excision of pilonidal sinus.

The main limitation of our study is its uni-centeredness, so the results are dependent on the skills of operating teams within the institution where the study was done. Cognitive status was assessed by the investigators on clinical grounds, instead of conducting Mini Mental test, so certain degree of subjectivity could not be ruled out. Besides, follow-up of the patients was limited to their hospital stay, since we lacked resources to follow the patients at their homes or in outpatient facilities.

Conclusion

Our study showed that minor elective surgical interventions (elective laparoscopic cholecystectomy, open inguinal hernia repair or excision of pilonidal sinus) are associated with only moderate (less than 25%) and short (one day) immediate postoperative decrease in quality of life, which is followed by increase in quality of life on discharge from hospital to the levels higher than preoperative.

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Flexural strength and modulus of autopolimerized poly(methyl methacrylate) with nanosilica

Savojna čvrstoća i modul elastičnosti autopolimerizovanog polimetilmetakrilata sa nanočesticama silicijum-dioksida

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Abstract

Background/Aim. Autopolymerized, or cold polymerized poly(methyl methacrylate) class of materials have a lower mechanical properties compared to hot polymerized poly(methyl methacrylate), due to a limited time of mixing before the polymerization process begins. The aim of this study was to test the effect of different relatively low nanosilica contents, in improving mechanical properties of the cold polymerized poly(methyl methacrylate). **Methods.** A commercially available autopolymerized poly(methyl methacrylate) denture reline resin methyl methacrylate liquid component was mixed with 7 nm after treated hydrophobic fumed silica and subsequently mixed with poly(methyl methacrylate) powder. Three nanosilica loadings were used: 0.05%, 0.2% and 1.5%. Flexural modulus and strength were tested, with one way ANOVA followed by Tukey's test. Furthermore, zeta potential, differential scanning calorimetry, scaning electrone microscopy and energy dispersive X-ray analyses were performed. **Results.** Flexural modulus and strength of poly(methyl methacrylate) based nanocomposites were statistically significantly increased by the addition of 0.05% nano-SiO2. The increase in nanosilica content up to 1.5% does not contribute to mechanical properties tested, but quite contrary. The main reason was agglomeration, that occurred before mixing of the liquid and powder component and was proved by zeta potential measurement, and after mixing, proved by scanning electrone microscopy and energy dispersive x-ray analyses. **Conclusions.** Addition of 7 nm 0.05% SiO_2 is the most effective in increasing flexural modulus and strength of autopolimerized poly(methyl methacrylate).

Key words:
methylmethacrylate; acrylates; denture rebasing; materials testing; stress, mechanical; nanoparticles; silicon dioxide; elasticity; calorimetry, differential scanning.

Apstrakt

Uvod/Cilj. Autopolimerizujući ili hladno polimerizujući polimetilmetakrilatni materijali imaju niže mehaničke osobine u odnosu na toplo polimerizujuće polimetilmetakrilate, zbog ograničenog trajanja mešanja pre početka procesa polimerizacije. Cilj ovog rada bio je da se ispita efekat relativno niskih sadržaja nanosilike u cilju povećanja mehaničkih osobina hladno polimerizujućeg polimetilmetakrilata. **Metode.** U tečnu metilmetakrilat komponentu komercijalnog autopolimerizujućeg polimetilmetakrilatnog materijala za podlaganje zubnih proteza umešane su nanočestice veličine 7 nm, a potom je modifikovana tečnost pomešana sa prahom polimetilmetakrilata. Ispitana su tri sadržaja nanosilike: 0,05%, 0,2% i 1,5%. Izvršena su ispitivanja modula elastičnosti i čvrstoće. Rezultati su statistički analizirani uz primenu jednostruke statističke analize ANOVA i Tukey-testom. Takođe, izvršeno je merenje zeta potencijala tečne komponente, diferencijalna skenirajuća kalorimetrija, skenirajuća elektronska mikroskopija i energetska disperzivna rentdenska analiza. **Rezultati.** Modul elastičnosti i čvrstoća bili su statistički značajno povećani dodatkom 0,05% nano-SiO_2. Povećanje sadržaja nanosilike na 1,5% nije doprinelo povećanju ispitivanih mehaničkih osobina, naprotiv. Osnovni razlog bio je pojava aglomeracije, pre mešanja praha i tečne komponente, dokazana merenjem zeta-potencijala, kao i nakon mešanja, a dokazana je skenirajućom elektronskom mikroskopijom i energetskom disperzivnom rendgenskom analizom. **Zaključak.** Najefikasniji sadržaj nanosilike za povećanje modula elastičnosti i čvrstoće autopolimerizovanog polimetilmetakrilata je 0,05 %.

Ključne reči:
metilmetakrilati; akrilati; zubna proteza, podlaganje; materijali, testiranje; stres, mehanički; koloidi; silicijum dioksid; elastičnost; kalorimetrija.

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Introduction

Poly(methyl-methacrylate) (PMMA) is one of the most widely used polymer materials due to the convenient combination of properties such as transparency, aesthetics, and biocompatibility. Such properties enabled it to be used for denture bases, of which, the vast majority are made of PMMA [1]. A special type of PMMA is autopolymerized, that is, it is synthesized by the successive addition of free radical building blocks. Free radicals can be formed via separate initiator molecules formed by the reaction between the materials powder and liquid components [1, 2]. The autopolimerized PMMA has been widely used to provide better retention of removable prostheses in cases of alveolar resorption, as well as for denture reparation in case of crack or fracture [3]. However, their mechanical properties are lower if compared with the heat-polymerized PMMA used for denture bases [4]. The main reason is a higher amount of unconverted monomer which acts as a microvoid. This causes stress concentration which can initiate internal or external cracks making the material less resistant [5, 6]. There are various methods of improving the mechanical properties of autopolimerized MMA such as: heat post-treatment, which can be achieved by hot water treatment [7] and by microwave post-irradiation [8–11]. These methods can be used only when the autopolimerized PMMA material is already applied to the denture, so the heat input may cause the denture to deform, that is, change its shape and therefore become unsuited to the geometrical configuration of the patients mouth [12]. An alternative approach is the introduction of rods or particles in the material, to obtain a composite material that would exhibit an increase in mechanical properties compared to the autopolymerized PMMA.

In the work by Carlos and Harrison [13] ultra high molecular weight polyethylene (UHMWPE) was added to the PMMA to reinforce added to acrylic resin denture base material. However, the results showed that impact strength and hardness were reduced. The studies by Chow et al.[14, 15] showed that the addition of hydroxyapatite at maximum content of 5% can increase fracture toughness. At the same time, flexural strength decreased, most probably due to agglomeration of the particles.

The addition of up to 15% of ZrO_2 can significantly increase the impact strength of the PMMA [16]. A study by Ellakwa et al. [17] showed that Al_2O_3 addition can significantly increase flexural strength of PMMA. These studies led to the tests done by Alhareb et al. [18], where various Al_2O_3/ZrO_2 particle ratios in the PMMA matrix showed a positive effect in increasing flexural strength and modulus as well as fracture toughness, however, tensile strength and modulus were reduced. Obviously, the particle type, size, content, surface properties and distribution all influence the composite mechanical properties. An approach used in works by Balos et al. [19, 20] comprised of the addition of relatively low (under 2%) of hydrophobic SiO_2 particles to improve the mechanical properties (microhardness, flexural strength, flexural modulus and fracture toughness) of flow dental composites, as well as PMMA denture bases. Low SiO_2 particle addition of 0.05% proved to be beneficial for all tested mechanical properties. The main reason was a lower agglomeration and more convenient distribution on reinforcing particles.

The aim of this study was to test the effect of different contents of relatively low nanoparticle content, added to the autopolymerized PMMA material, and to determine mechanical properties of nanocomposite, in terms of modulus and flexural strength.

Methods

The material used in this study was a commercial PMMA denture reline resin Simgal (Galenika, Zemun, Serbia), supplied separately in powder and liquid. The powder consisted of the PMMA, benzoyl peroxide and inorganic pigments, while the liquid being the MMA monomer and the tertiary amine. Benzoyl peroxide and tertiary amine initiate the radical polymerization process. Samples were prepared in accordance with manufacturers instruction, with powder to liquid ratio of 2 : 1 in weight. PMMA was modified with hydrophobic properties: AEROSIL R812 (Evonik, Essen, Germany) 7 nm SiO_2 with hydrophobic properties and specific surface area of between 195 and 245 m^2/g. Nanoparticles were weighed on analytical balance (Adventurer Pro Ohaus, Parsippany, NJ) with an accuracy of 0.0001 g and subsequently mixed with the liquid component by using a magnetic stirrer MM-530 (Tehtnica, Zelezniki, Slovenia) for 10 min. Afterwards, the ultrasonic bath PS-20A (Challenger, Selangor, Malaysia) was used for 10 min, to prevent agglomeration, while the final mixing was done in magnetic stirrer again for 2 min, to obtain stabilized solution of nanoparticles in the liquid component. To determine the size of the particles in the liquid component, Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) analyzer was applied. The liquid component was then mixed with powder component and the obtained mix was poured into square Al-alloy molds. Then, after polymerization, a set of silicone grit papers (150, 400 and 1200-grit) was used to get the desired shape and dimensions of the samples. The dimensions were verified by a micrometer, accurate to 0.01 mm at 3 locations. Five specimens were used for each testing, for each of the following sample groups: control group (unmodified), the group with 0.05, 0.2 and 1.5 (wt.)% nanoparticles in relation to the overall weight of the liquid and powder component.

The flexural modulus and flexural strength were determined by using an AT-L-118B (Toyoseiki, Tokyo, Japan) tensile testing machine, with a crosshead speed of 50 mm/min. 3 point bending test was used, with the distance between the supports of 40 mm. Specimens dimensions were 6 x 2.5 x 45 mm.

The flexural modulus was calculated by using the following equation:

$$E = \frac{\Delta F l^4}{4 \Delta d b h^3} \quad (1)$$

where l is the distance between the supports [mm], Δd is the displacement range [mm] for a given testing load range ΔF

[N], b is specimen width [mm] and h is specimen height [mm].

The flexural strength was calculated by using the following equation:

$$\sigma = \frac{3Fl}{2bh^2} \quad (2)$$

where F is maximum force [N], l is the distance between the supports [mm], b is specimen width [mm] and h is specimen height [mm].

To evaluate the significance between the results of mechanical properties of different sample groups, one-way analysis of variance (ANOVA) followed by Tukey's test with the significance value of $p < 0.05$ was used. The tests were performed by using Minitab 16 software.

To determine thermal properties of obtained materials, differential scanning calorimetry (DSC) analysis was performed. Q20 (TA Instruments, New Castle, DE) DSC device was applied, in the temperature range from 60°C to 160°C, with a scan rate of 10°C/min. The glass transition temperatures were determined on the basis of the second heating.

The fracture surfaces were examined by JSM-6460LV (JEOL, Tokyo, Japan) scanning electron microscope (SEM), operating at 25 kV, both in secondary electrone and backscattering electrone mode. The specimens were previously coated with gold, using the SCD-005 (Bal-tec/Leica, Wetzlar, Germany) device. Furthermore, to examine particles found on fracture surfaces, energy-dispersive X-ray spectroscopy (EDX) was used.

Results

The size distribution of particles in the liquid component of the PMMA material used in this study is shown in Figure 1. General trends are similar to single peak shaped distribution in liquid components used for obtaining specimens with 0.05% and 0.2% nanosilica. In the liquid specimen used for obtaining the specimen with 1.5% nanosilica, 2 peaks can be seen, with a secondary peak set at 52 nm particle size. Also, the only eqisting (0.05% and 0.2% SiO_2) and primary peak (1.5% SiO_2) was found at slightly elevated particle size as the content of particles increases.

The flexural modulus and strength, standard deviations and the results of statistical analysis of control and modified specimens are shown in Figures 2 and 3. It can be seen that the mechanical properties vary in accordance to the nanoparticle type added. SiO_2 7 nm particles offered a significantly increased flexural modulus at 0.05% and strength at 0.05% and 0.2% contents. The highest flexural modulus and strength were obtained with the lowest, 0.05% nanoparticle loading. At 1.5% loading, mechanical properties were lower compared to control specimen group. The statistical difference between the modified specimen (1.5% SiO_2) in terms of flexural modulus was significant compared to the control specimen, without nanoparticles added. However, in terms of flexural strength this difference was not significant.

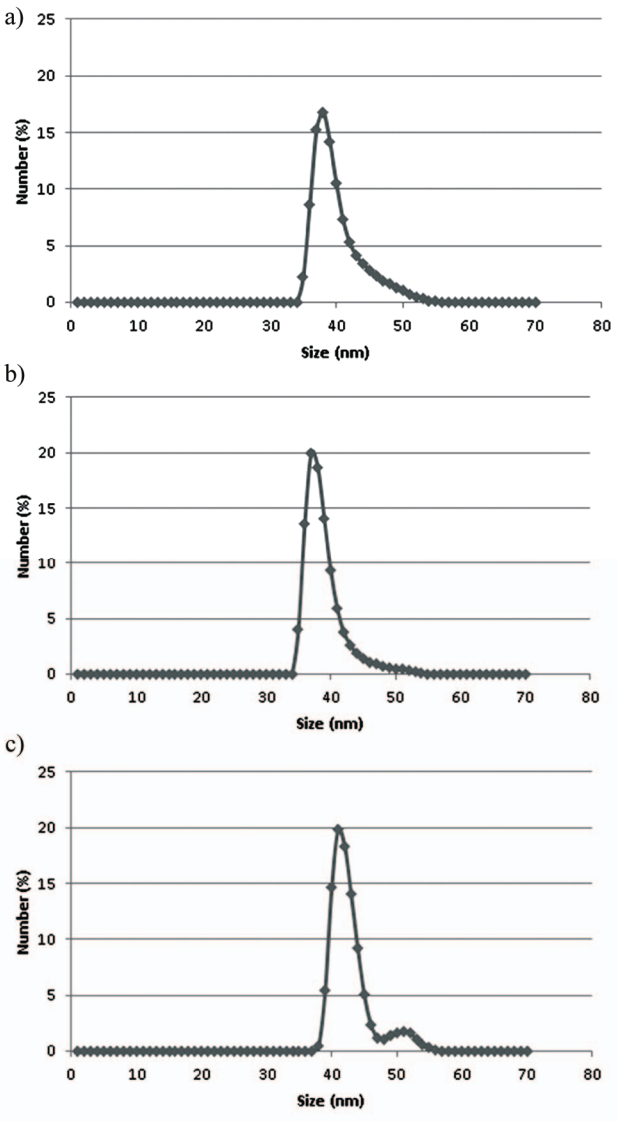

Fig. 1 – Particle size in liquid component with different contents of nonosilica: a) 0.05%; b) 0.2%; c) 1.5%.

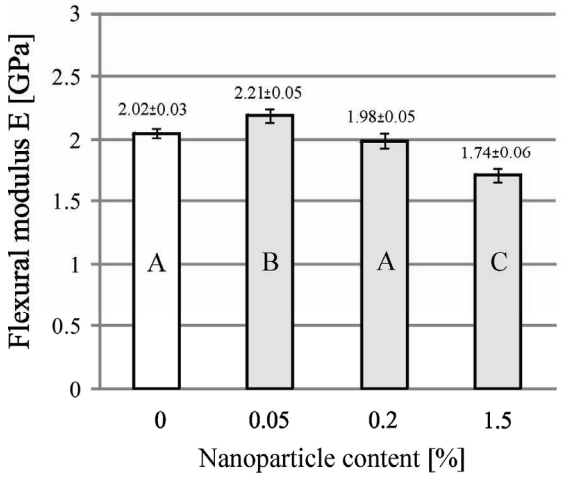

Fig. 2 – Flexural modulus and standard deviations of tested materials. Different letters indicate statistically significant differences at a level of 95%.

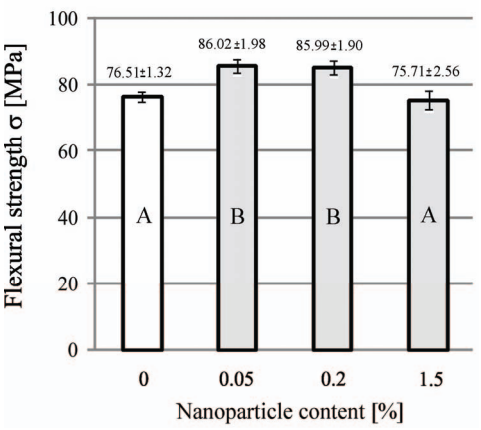

Fig. 3 – Flexural strength and standard deviations of tested materials. Different letters indicate statistically significant differences at a level of 95%.

Differential scanning calorimetry (DSC) curves shown in Figure 4 reveal that the addition of nanoparticles increase glass transition temperatures (Tg) at all contents. The increase in the Tg was moderate and ranges up to 5°C. The highest increase is in specimen groups modified with 0.05% and 0.2% SiO_2.

The representative fracture surfaces are shown in Figure 5. It can be seen that a brittle fracture mode was present in all specimens, with a characteristic river pattern. Furthermore, the fracture surface showed that the crack propagated between basic material powder particles (Figure 5a, d) and partially through them (Figure 5b, c).

Another feature of the fracture surfaces was the presence of agglomerates, (Figure 6). In Figure 6, SiO_2 agglomerate was shown in secondary electron SEM mode, as degradation of the polymer matrix underneath the agglomerate during the energy disperisive X-ray (EDX) testing process.

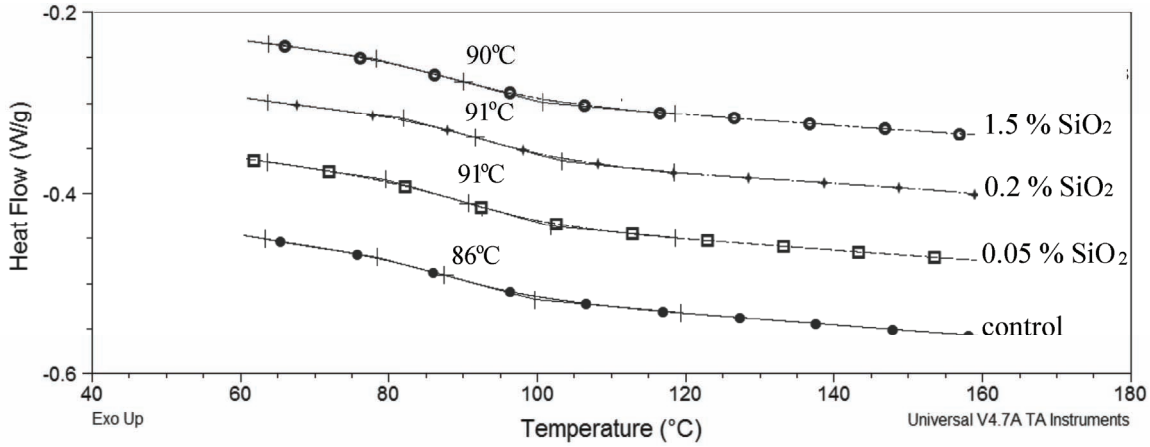

Fig. 4 – Differential scanning calorimetry (DSC) curves of prepared samples with different type and content of nanoparticles.

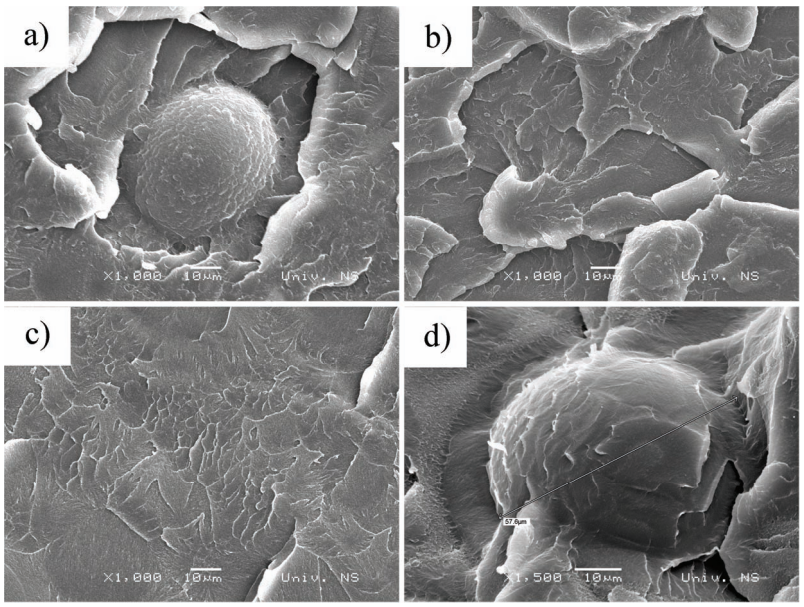

Fig. 5 – Fracture surfaces scanning electron microscope (SEM): a) control specimen; b) 0.05% SiO_2; c) 0.2% SiO_2; d) 1.5% SiO_2.

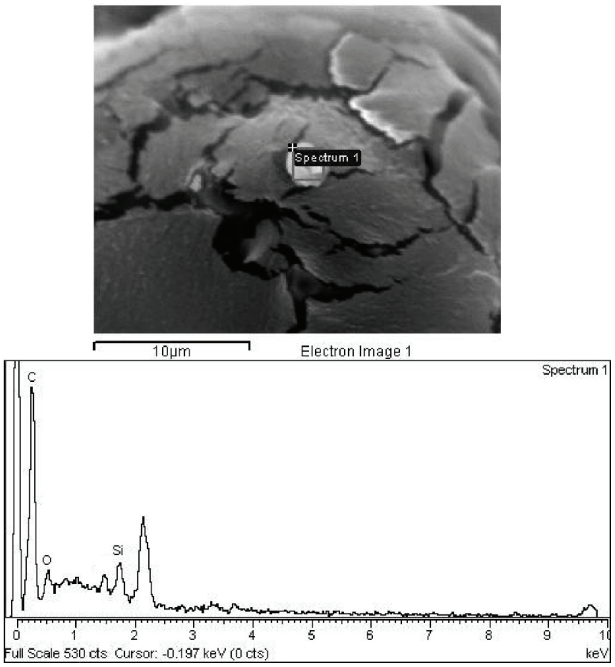

Fig. 6 – Energy dispersive X-ray (EDX) analysis of 1.5 % SiO_2 added to a poly (methyl-methacrylale) specimen.

Discussion

The results presented in this paper show a strong correlation between flexural modulus, flexural strength, glass transition temperatures (Tg), fracture surfaces and EDX analysis. Various investigations suggest that Tg of nanocomposites vary depending on different factors, such as the type of polymer matrix, type and size of nanoparticles and method of preparation [21–23]. The change in the Tg value of the composite is mainly associated with the immobility of the polymer macromolecules in the interfacial layer within few nanometers of the nanoparticle surface. If the interfacial layer is thin and a small amount of polymer is immobilized, the change in the Tg value is not expected [24, 25]. The ideal polymer nanocomposite material has a well dispersed nanoparticles with sufficiently small distances between them, so interfacial layers contact each other forming a homogenous reinforced field [23]. Interfacial layer thickness can be calculated using the following equation (3) [24]:

$$D = \frac{d_p}{2}\left[\sqrt[a]{\left(a_d \frac{1-y_p}{y_p}\right)} - 1\right] \qquad (3)$$

where: d is the interfacial layer thickness, d_p is the nanoparticle diameter, a_d is the fraction of monomer (polymer) that forms the interfacial layer and y_p is the nanoparticle content in the nanocomposite [24]. Equation 3 shows that the interfacial layer thickness is directly proportional to the nanoparticle diameter. The results shown in this paper suggest that mechanical properties (flexural modulus and strength) are proportional to Tg. However, in specimens containing 1.5(wt.)% of SiO_2 nanoparticles, Tg is lower compared to other nanoparticle contents. This result suggests that agglomeration occurs. Namely, as agglomerates are considerably larger than nanoparticles (shown in Figure 5), their larger size can cause the increased thickness of interfacial layer. However, as the overall number of agglomerates is considerably lower than the overall number of nanoparticles, the impact of a larger number of interfacial layers may have beneficial effect on increasing the Tg. This finding is supported by the detection of relatively large agglomerates compared to initial particle size in the higher nanoparticle content (1.5%). Also, the deterioration in mechanical properties was generally obtained with the highest nanoparticle content of 1.5%, which can indicate that agglomerates affect the mechanical properties. These results are supported by previous investigations [19, 20]. Agglomerates influence the drop in mechanical properties in several ways. The most obvious is the decrease in the effective number of reinforcing particles, causing gaps between the interfacial layers (reinforced fields) [19, 20, 25]. This is confirmed with the results of fracture surface examination. In specimens containing 1.5% SiO_2 fracture surfaces, as well as, in control specimens, the crack propagates between powder particles, while in other specimen groups, the crack propagates through the powder particles. This indicates that the inter powder particle polymer material, polymerized during material synthesis in the control and 1.5% SiO_2 specimen groups is significantly less resistant compared to powder material, so the crack propagates through it, rather than through powder particles. This correlates well to flexural modulus and strength obtained with the control and 1.5% SiO_2 content specimen groups. Another negative effect of higher nanoparticle contents is that large agglomerates can fracture under load, while their fragment or fragments can remain firmly bonded to the PMMA matrix, that is, to the interfacial layer. It must be noted that agglomerates fracture at lower local stresses than nano or micro particles of the same size. The main reason for such behavior is their relatively low cohesive strength, due to the presence of relatively weak secondary bonds (Van der Waals forces, hydrogen, capillary, or by adsorption of foreign substances) between individual nanoparticles [26, 27], compared to the ionic or covalent bonds within ceramic fillers. After agglomerate fracture, the stress is suddenly transmitted to the matrix, causing matrix overload and premature failure [20, 28].

Conclusion

The incorporation of 7 nm SiO_2 nanoparticles into autopolimerized poly(methyl methacrylate) is beneficial for improving flexural modulus and strength. The most effective content is 0.05% SiO_2. The highest applied concentrations of 1.5% are proved to be ineffective to the level that flexural modulus and strength are lower than that of the unmodified specimen. Agglomeration plays an important role in reducing mechanical properties compared to the control unmodified specimens.

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The effects of local endometrial injury made by hysteroscopy on *in vitro* fertilization outcome

Uticaj lokalne endometrijalne povrede načinjene u toku histeroskopije na ishod vantelesne oplodnje

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Abstract

Bacngraund/Aim. Regardless of embryos quality, an appropriate endometrial thickness and a successful embryo transfer (ET)**,** implantation remains a difficulty for a favorable outcome of an *in vitro* fertilization (IVF). Numerous studies with the aim of increasing implantation rate and pregnancy rate suggest local endometrial injury (LEI) prior to the IVF procedure. Hysteroscopy is a method becoming more widely used both with patients after a failed IVF cycle and with patients before the first IVF, considering the large incidence of uterus cavum pathological states which have a negative impact on the favorable outcome. However, there is still no consensus reached over LEI, the way and time of its performing or its impact. The aim of this research was to determine the effect of hysteroscopically made local endometrial lesion on the IVF procedure outcome, both in the first and in the next IVF cycle and also to examine if the new method of LEI provoking using bipolare electrode has a favorable impact on post IVF pregnancy success. **Methods.** Total of 81 patients had hysteroscopy performed 30–50 days prior to IVF, 40 of which had LEI made during hysteroscopy (the LEI group) using bipolar electrode in a way not described in any available literature. Remaning hysteroscopically treated patients (n = 41) had no LEI (the non-LEI group). The control group included 151 patient who had IVF performed with no prior hysteroscopy and LEI. **Results.** The rate of clinical pregnancies after LEI was statistically more significant in comparison to the control group (52.50% *vs* 34.44%, $p < 0.05$) and it meant 2.1 time bigger chance to conceive (OR=2.10; 95% CI: 1.04 – 4.26; $p < 0.05$). We noticed differences in the implantation rate between the LEI group and the non-LEI group on one side and the control group on the other, in favor of the two groups subjected to hysteroscopy (23.89%, 25.47% vs 18.18%, respectively), but they were not of any statistical significance. Not significantly higher rate of pregnancy was present, after the first and the next IVF, both in the LEI and the non-LEI group when compared to the control one. **Conclusion.** New method of LEI provoking by bipolar electrode in the process of hysteroscopy is a simple and safe method allowing higher rate of clinical pregnancies and doubling the probabilty of the positive IVF outcome.

Key words:
infertility; fertilization in vitro; hysteroscopy; iatrogenic disease; uterus; wounds and injuries; pregnancy outcome.

Apstrakt

Uvod/Cilj. Bez obzira na kvalitetne embrione, odgovarajuću debljinu endometrijuma i uspešan embriotransfer (ET), implantacija ostaje problem za povoljan ishod vantelesnog oplođenja (VTO). Histeroskopija je metoda koja se sve više koristi kako kod pacijentkinja nakon neuspešnog ciklusa VTO tako i kod pacijentkinja pre prve VTO, imajući u vidu veliku incidencu patoloških stanja kavuma uterusa koja negativno utiču na povoljan ishod. Brojne studije u cilju povećanja stope implantacije i stope trudnoća predlažu lokalnu endometrijalnu povredu (LEI) pre postupka VTO, međutim, i dalje ne postoji konsensus oko LEI, načina i vremena izvođenja i njenog uticaja. Cilj ovog istraživanja bio je da se utvrdi efekat histeroskopski načinjene lokalne LEI na ishod VTO postupka, kako kod prve VTO tako i kod naredne, a takođe i da se ispita da li novi način izazivanja LEI pomoću bipolarne elektrode ima povoljan uticaj na ostvarivanje trudnoća nakon VTO. **Metode.** Kod 81 pacijentkinje histeroskopija je rađena 30–50 dana pre VTO, od kojih je kod 40 pacijentkinja načinjena LEI u toku histeroskopije bipolarnom elektrodom, (LEI grupa) na način koji nije opisan u dostupnoj literaturi. Kod preostale 41 pacijentkinje nije izvršena LGI (*non* LEI grupa). Kontrolna grupa (II grupa) je obuhvatila 151 pacijentkinju kod koje je rađen VTO bez prethodne histeroskopije i LEI. **Rezultati.** Utvrđena je statistički značajno viša stopa kliničkih trudnoća nakon LEI u odnosu na kontrolnu grupu

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(52.50% *vs* 34.44%, $p < 0.05$) i 2,1 put veća šansa da se ostvari trudnoća (OR = 2,10; 95% IP: 1,04–4,26; $p < 0.05$). Utvrdili smo razliku u stopi implantacije između LEI i *non* LEI grupa s jedne i kontrolne grupe s druge strane (23,89%, 25,47% *vs* 18,18%, redom), ali bez statističke značajnosti. Bila je prisutna, ali ne značajno viša, stopa trudnoća i nakon prve VTO i nakon sledeće u LEI i *non* LEI grupi u odnosu na kontrolnu grupu. **Zaključak**. LEI načinjena bipolarnom elektrodom u toku histeroskopije je jednostavna i sigurna metoda koja daje višu stopu kliničkih trudnoća i duplira verovatnoću za pozitivan ishod VTO.

Ključne reči:
neplodnost; oplođenje in vitro; histeroskopija; jatrogena bolest; materica; povrede; trudnoća, ishod.

Introduction

Implantation represents a critical phase in *in vitro* fertilization (IVF) cycle. Despite having a good number of oocytes and embryos quality as well as an appropriate thickness of the endometrium, in the last decade, pregnancy rate was the same - about 30%–40% in the IVF cycle.

The idea of performing hysteroscopy prior to every IVF procedure was greatly favored for two reasons: due to high frequency of uterus cavum pathological states (10%–60%) and the influence of hysteroscopy itself on the better IVF outcome [1, 2].

Many studies have tried to show that local endometrial injury (LEI) prior to IVF leads to an inflammatory response causing an increased endometrial receptivity and bigger IVF success rate [3–5]. In the majority of studies, LEI was made on the front or the back of uterus wall [6], using Pippele biopsy catheter [7–9], Novak biopsy catheter [10] or endometrial cell sampler [11]; in smaller number of studies, LEI was made during hysteroscopy using grasping forceps [12, 13] or monopolar electrode [14].

However, there is still no agreement on the necessity of a mechanical endometrial injury (EI), on the way and the timing of its making, neither on whether it is better for the EI to be made by biopsy or during diagnostic hysteroscopy.

For all these reasons, the aim of this study was to determine the influence of LEI on IVF during hysteroscopy and whether provoking an endometrial lesion by using bipolar electrode can favorably influence on the IVF outcome before the first IVF cycle as well as on the next.

Methods

The research was done at the Clinic of Gynecology and Obstetrics of the Clinical Center in Niš as a prospective study and it included 232 patients from the National IVF program with maximum of 2 IVF cycles on the state's budget.

Criteria for being included in the research were: less than 40 years of age, FSH < 15 IU/mL, anti-Müllerian AMH > 0.5, body mass index (BMI) < 30 kg/m², lack of genital infection and favorable caryotype of both partners.

Criteria for being excluded were: presence of chronic systemic disease, existence of hepatitis C or human immunodeficiency virus (HIV) infection, organic pathology of ovaries, immune sterility cause and azoospermia.

The patients were divided into 3 groups: the group I of 40 patients who had hysteroscopy and LEI performed prior to IVF – the LEI group; the group II of 41 patients, with hysteroscopy prior to IVF but with no LEI – the non-LEI group; and the control group (the group III) of 151 patients with no hysteroscopy nor LEI. All the patients from the groups I and II had a favorable hysteroscopical report.

Hysteroscopy was performed during oral contraceptive therapy, 30–50 days before IVF. Saline solution was used as a distension medium and 5 mm Bettocchi office hysteroscop (Karl Storz GmbH and co, Tuttlingen, Germany) with 5Fr working canal. The patients were in a lithotomyc position with short-term intravenous anesthesia applied. Vaginoscopic approach was used with no cervix traction. LEI was made in a way which was not described in any literature available. It was performed with a springle bipolar electrode (Johnson), fundic, in a transversal direction, 10–15 mm in length, throughout the whole endometrial thickness.

The IVF procedure was performed 1–2 months after oral contraceptive therapy. A long and short protocol with gonadotropin-releasing hormone (GnRH) agonists was used. Serial ultrasound checkups during controlled ovary hyperstimulation (COH) were done with a Shimadzu ultrasound device, starting on the 6th day of stimulation. On finding 2 or more follicles larger than 18 mm, patients got 10,000 U Pregnyl® injection and a transvaginal oocytes pick-up (OPU) was performed 34–36 hours afterwards. Embryo transfer (ET) was done on the day 2, 3 or 5 after the aspiration, monitored by an ultrasound, putting back a maximum of 3 embryos Cook's catheter was used for the ET. After the ET, the patients underwent the following therapy: tabl Utrogestan® 200 mg, 3 times a day, vaginal application; tabl Cardiopirin® 100 mg, once a day; tabl Dexason® 0.5 mg, once a day. 10–12 days post ET (15 days post OPU), the ß-subunit of human chorionic gonadotropin (ßHCG) from blood was determined for biochemical verification of pregnancy. Clinical pregnancies were verified by transvaginal ultrasound checkup by visualization of the embryo's cardio activity 4–5 weeks after the ET.

This prospective clinical trial was approved by the Ethics Committee of the Clinical Centre in Niš. The treatment of the patients included hysteroscopy and a long and short GnRH agonist protocol. Written informed consent was provided by all patients participating in the study.

The data were processed by using standard descriptive statistical methods (average value, percentage representation). The statistical processing was done among defined groups. Continual variables relative to data distribution were compared using Student's *t*-test, Pearson's χ^2 test or ANOVA

test. Also, a univariate logistic regression analysis was used for determination of important IVF outcome parameters.

Results

The patients from the examined groups were not significantly different in any of the generally examined parameters (Table 1).

There were also no statistically significant differences among the groups considering the number of oocytes, conceived embryos, transferred embryos and the day of embryo transfer (Table 2). The long protocol with agonists was most frequently used with all 3 groups of the patients. Based on the general parameters and the features of the IVF cycle, homogeneity of the groups was present making the further results valid for this research.

The implantation rate was higher in the non -LEI group (25.47%) and in the LEI group (23.89%), when compared to the control one (18.18%), but with no statistical significance.

Clinical pregnancy rate was highest in the LEI group; it was significantly higher in comparison to the control group (52.50% *vs* 34.44%, $p < 0.05$). A difference was also noticeable between the LEI and non-LEI group (52.50% *vs* 46.34%), but with no statistical significance. The same was noticed between the non-LEI group and the control one.

There was no statistically significant difference neither in the multiple pregnancy rate nor in the rate of biochemical pregnancy, comparing the 3 groups (Table 2).

The rate of pregnancy was higher after the second IVF in the LEI group, whereas in the non LEI group and the control group, the first IVF showed better results, although with no statistically significant difference (Table 3).

Due to considerably more frequent long protocol with agonists, we compared pregnancy rates in relation to the applied stimulation protocol. Higher rate of pregnancy was present in the LEI group with long protocol than in the control group, and also in the non-LEI group with short protocol; these differences were not statistically significant. This leads to a conclusion that application of a certain protocol cannot seriously influence obtained results (Table 3).

Table 1

General characteristics of patients in the examined groups

Parameters	LEI group (n = 40)	non LEI group (n = 41)	Control group (n = 151)
Age (years)	32.78 ± 4.12 (32.50)	34.00 ± 3.49 (35.00)	33.61 ± 3.65 (34.00)
Patients per age group (years), n (%)			
≤30	14 (35.00)	7 (17.07)	34 (22.52%)
31–35	14 (35.00)	19 (46.34)	61 (40.40%)
36–40	12 (30.00)	15 (36.59)	56 (37.09%)
Duration of infertility (years)	6.20 ± 3.08 (5.00)	5.93 ± 2.86 (6.00)	6.38 ± 3.58 (6.00)
FSH (mU/mL)	5.95 ± 2.10 (5.50)	6.88 ± 2.90 (6.10)	5.93 ± 2.59 (5.50)
AMH (ng/mL)	3.57 ± 2.39 (3.43)	2.92 ± 2.88 (1.84)	3.02 ± 2.47 (2.18)
BMI (kg/m²)	23.28 ± 2.91 (22.50)	23.59 ± 2.96 (23.00)	23.50 ± 2.95 (23.00)

Data are given as absolute numbers (percentages), or mean ± standard deviation (median);
LEI – local endometrial injury; FSH – follicle-stimulating hormone; AMH – anti-Mülleriar hormone;
BMI – body mass index.

Table 2

***In vitro* fertilization (IVF) cycle features of patients in the examined groups.**

Parameters	LEI group	non-LEI group	Control group
Gonadotropin (U)	2,113.00 ± 792.36 (2012.50)	2,078.66 ± 710.05 (1975.00)	2,019.97 ± 674.64 (1950.00)
Oocytes, n	10.35 ± 8.64 (10.00)	10.73 ± 7.37 (10.00)	9.98 ± 6.58 (8.00)
Embryos, n	5.88 ± 4.50 (5.00)	5.59 ± 3.54 (5.00)	4.89 ± 3.22 (4.00)
Transferred embryos, n	2,82 ± 0.15 (3.00)	2.58 ± 0.41 (3.00)	2.68 ± 0.20 (3.00)
Protocol, patients			
long agonists	33 (82.50)	27 (65.85)	98 (64.90)
short agonists	7 (17.50)	14 (34.15)	53 (35.10)
Endometrial thickness (mm)	10.88 ± 1.67 (11.00)	9.98 ± 1.57 (10.00)	10.02 ± 1.72 (10.00)
First / second IVF	24 (60.00) / 16 (40.00)	26 (63.41) / 15 (36.59)	108 (71.52) / 43 (28.48)
ET day	3.83 ± 1.03 (3.00)	3.34 ± 0.94 (3.00)	3.65 ± 0.98 (3.00)
2nd	1 (2.50)	4 (9.76)	4 (2.65)
3rd	22 (65.00)	28 (68.29)	96 (63.58)
5th	17 (32.50)	9 (21.95)	51 (33.77)
Implantation rate	23.89% (27/113)	25.47% (27/106)	18.18% (72/396)
Clinical pregnancy rate	52.50% (21/40*c)	46.34% (19/41)	34.44% (52/151)
Biochemical pregnancy rate	10.00% (4/40)	12.20% (5/41)	6.62% (10/151)
Multiple pregnancy rate	23.81% (5/21)	36.84% (7/19)	36.54% (19/52)

Data are given as absolute numbers (percentages), or mean ± standard deviation (median);
LEI – local endometrial injury; ET – embryo transfer; *$p < 0.05$, c – *vs* control group.

Table 3

Succes rate (pregnancy) for first and second *in vitro* fertilization (IVF) and the applied protocol

Parameter	Succes rate (SR)		
	LEI	non-LEI	Control
First VTO [SR = 65 (41.14%)]			
patient, n	24	26	108
pregnancy, n (%)	12 (50)	13 (5)	40 (37.04)
Second IVF [SR = 27 (36.49%)]			
patient, n	16	15	43
pregnancy, n (%)	9 (56.25)	6 (40.00)	12 (27.91)
Long protocol with agonist [SR = 68 (43.04%)]			
patient, n	33	27	98
pregnancy, n (%)	18 (54.55)	12 (44.44)	38 (38.78)
Short protocol with agonist [SR = 24 (32.43%)]			
patient, n	7	14	53
pregnancy, n (%)	3 (42.86)	7 (50.00)	14 (26.42)

LEI – local endometrial injury.

Table 4

Estimation of the influence of important factors on conceiving – results of a univariate logistic regression analysis

Factor	*p*	OR	95% CI for OR boundary	
			Lower	Upper
LEI group	0.0388*	2.10	1.04	4.26
non- LEI group	0.1636	1.64	1.82	3.31
Age (years)	0.0079**	0.91	0.84	0.97
Age group, 36–40 years	0.0321*	0.46	0.23	0.94
Oocytes	0.0280*	1.04	1.00	1.08
Embryos	0.0055**	1.11	1.03	1.19
Endometrial thickness	0.0160*	1.22	1.04	1.43
IVF/ICSI	0.0243*	0.48	0.25	0.91

*** – $p < 0.05$; ** – $p < 0.01$, *** – $p < 0.001$**

OR – odds ratio; CI – confidence interval; LEI – local endometrial injury; IVF – *in vitro* fertilization; ICSI – intra-cytoplasmic sperm injection.

A univariate logistic regression analysis proved that the age as a continual variable, the age group 36–40, the number of obtained oocytes, the number of conceived embryos, the IVF in relation to intra-cytoplasmic sperm injection (ICSI) and the LEI in relation to the control group, were statistically important predictors of the pregnancy outcome (Table 4).

The LEI group of patients had statistically significant, 2.1 time bigger chance for pregnancy when compared to the control group [odd ratio (OR) = 2.10; 95% confidence interval (CI): 1.04–4.26; $p < 0.05$), but with no statistically significant difference when compared to the non-LEI group of patients. The non-LEI group of patients, in comparison to the control group, had a higher probability of pregnancy (64%), but it was not of statistical significance (OR = 1.64; 95% CI: 0.82–3.31).

Discussion

Mechanical LEI before ovary stimulation for IVF was suggested in order to improve the conditions for implantation in women having inexplicable repeated implantation failure (RIF). It has been shown that mechanical manipulation on the endometrium can improve the receptivity in a way that it modulates the genes expression of factors necessary for the implantation, such as: glycodelin A, laminin alpha 4, integrin alpha 6 and matrix metalloproteinase 1 [2]. Mechanical manipulation or LEI can be provoked by endometrial biopsy or by hysteroscopy. It is assumed that due to the endometrial injury, changes occur in the endometrium, in the immune system and in the gene expression, all of which leads to increased receptivity and formation of an appropriate environment for implantation.

In their study, Gnainsky et al. [4] proved that endometrial biopsy provokes an inflammatory response characteristic for the influx of the macrophage and dendritic cells (DC), together with an increase of proinflammatory cytokines. Proinflammatory cytokines, produced in an injured endometrium, stimulate the secretion of chemokines and cytokines which later recruit the macrophage and DC cells to the implantation spot. These immune cells enforce the inflammatory reaction and provoke a synthesis of molecules in the endometrium which react on their own with a blastocyst, enabling its apposition and adhesion to the uterine wall.

Our research included LEI during hysteroscopy using a bipolar springle electrode, fundic in a transversal line, 10–15 mm in length. We did not find any data in literature saying that LEI was done in that way. The advantage of making an endometrial lesion in this way is that hysteroscopy is be-

coming more and more frequently used method, superior in diagnosing the states of uterus cavum. Due to frequent unexpected pathological results with patients having clear ultrasonography and hysterosalpingography (HSG) and also due to the possibility of making a surgery in a single act with extremely rare complications while performing hysteroscopy, it becomes significantly important as a procedure. Bipolar springle electrode is easy to handle and use. Having a larger circumference than the Twizzle® bipolar electrode, it enables a larger space of effect, there is no bleeding during intervention, there is no electricity overflow towards the tissue as with the monopolar electrode, no thermal effect further than the tissue contact spot, so it could be performed in daily hospital conditions.

Our study showed significantly higher rate of pregnancy with patients who underwent LEI in comparison to the control group (52.50% *vs* 34.44%; $p < 0.05$). We could not reach a statistical significance in comparison to the non- LEI group, possibly due to the sample size, even though there was a difference in favor of the LEI group (52.50% *vs* 46.34%).

In a retrospective study of Kara et al. [7], in which the endometrial injury was made by a biopsy catheter, on the day 21 of the cycle, prior to the IVF procedure, statistically higher rate of pregnancy was obtained in the study group when compared to the control group (43.9% *vs* 21.4%). One more research showed statistically significantly higher rates of implantation, clinical pregnancies and infants born alive in patients who underwent LEI within the cycle, prior to the IVF cycle [15]. The study group had rates of 13.07%, 32.7% and 22.4%, respectively; the rates of the control group were 7.1%, 13.7% and 9.8%, respectively. A pioneer study on 30 patients, in which, hysteroscopically, the endometrial injury was made with a jagged forceps on the *cavum* back wall, in the mid line, 10–15 mm from the *fundus*, showed the pregnancy rate of 100% in the LEI group and 46% in the group with no endometrial injury.

Unlike the former research, another study did not prove the benefit of LEI in the IVF procedure [8]. Rates of implantation, biochemical and clinical pregnancies in the experimental group were 4.9%, 18.2% and 12.1%, respectively; in the control group, they were 6.71%, 19.5% and 17.1%, respectively, without a statistically significant difference. Werner et al. [11] also stated that there was no improvement in the pregnancy rate with 39 patients who underwent a single endometrial biopsy within the cycle, prior to ET euploid frozen embryos, when compared to the control group (43.6% *vs* 55.0%; $p = 0.13$). One more study demonstrated no significant difference in ongoing pregnancy rates, implantation rates, clinical pregnancy rates and multiple pregnancy rates on 300 unselected subfertile women scheduled for IVF/ICSI treatment [8].

Karimzade et al. [16] discovered that LEI, on the day of oocytes pick-up in the IVF cycle, had a negative effect on the endometrial receptivity, causing triple lower rates of implantation and pregnancy in the experimental group in comparison to the control one.

Three latest studies which compared hysteroscopical groups with and without endometrial lesion, produced opposing results. Seval et al. [14] stated significantly higher rates of implantation and rates of pregnancy with the LEI groups, before IVF whereas El-Khayat et al. [13] and Shokeir et al. [12], examining the natural cycle and cycles with artificial insemination, did not find any justification to perform endometrial lesions.

The IVF success is decreasing with every following attempt. Therefore, pregnancy rates after the second and each following IVF cycle are significantly different from the first IVF, in each and every study. Our research also showed that the pregnancy rate after the second IVF in the LEI group was higher than after the first one, unlike in the non-LEI group in which there was only an insignificant difference in favor of the first IVF cycle. An interesting fact is that the pregnancy rate after the second IVF in the non- LEI group was higher than the pregnancy rate after the first IVF in the control group.

Statistical significance in pregnancy rate was also confirmed by a univariate logistic regression analysis, which pointed to the fact that patients had 2.1 time bigger chance of conceiving if LEI was done prior to the IVF cycle (OR = 2.10; 95% CI: 1.04–4.26; $p < 0.05$).

However, unlike other researches, we did not found a statistical significance in the implantation rate among the groups, although there was a difference between the groups I and II on one side and the control group on the other, in favor of the two first groups (23.89%, 25.47% *vs* 18.18%, respectively). The hysteroscopy itself definitely brings about a mild inflammatory response in the endometrium, leading in that way to an increase of implantation and clinical pregnancy rates which supports the idea of hysteroscopy being necessary even prior to the first IVF cycle and not only in cases of RIF with IVF program patients.

Result Limitation of this research is the smaller number of patients in the LEI and non- LEI group, which can make an impact on insufficient significance of one part of the results. To verify the method of making an endometrial lesion, a new group of patients should be introduced, to whom LEI would be performed using the usual procedure, i.e. with a biopsy catheter, which would enable comparison of effects of using different methods for making lesions.

Regardless of all the thorough research so far, no consensus has been reached on the use of hysteroscopy or endometrial biopsy, on the method of making a hysteroscopical injury, on a single or multiple biopsy or even on the intervention length in relation to the cycle stage.

Considering the frequency of unexpected pathological reports of the *cavum*, including prior the first IVF and after every failed IVF process, hysteroscopy should have an advantage over the biopsy catheter, bearing in mind the possibility of performing a surgical treatment on the changes detected in the diagnostic hysteroscopy. A major issue arouses: whether endometrial injury during hysteroscopy should be limited, i.e. to women with RIF or it could be applied to all the women participating in the IVF procedure, with no *cavum* pathology.

Conclusion

LEI made by a bipolar spingle electrode, fundic, in a transversal direction, in the process of hysteroscopical examination within the cycle prior to IVF, significantly increases the rate of clinical pregnancies and doubles the chance of conceiving post IVF. The increase of pregnancy rates can be seen with the first IVF cycle, but as well as with the next cycle, bringing about a dropping number of IVF attempts and also a shorter time needed for conceiving. LEI made by hysteroscopy represents a simple and safe method which can be performed even in outpatient conditions. It is necessary to conduct further research on a larger sample to verify the obtained results.

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Positive effects of hearing and speech rehabilitation on lexical range quality in hearing impaired children

Pozitivni efekti rehabilitacije sluha i govora na kvalitet leksičkog fonda dece sa oštećenim sluhom

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Abstract

Background/Aim. Initial experiences in rehabilitation of children with cochlear implants and frequent debates regarding the effects of their application have imposed the necessity to compare the effects of speech rehabilitation in children with hearing aids with those having cochlear implants. The aim of this study was to evaluate and compare the level of lexical development in hearing impaired children who are involved in the process of hearing and speech-language rehabilitation and who were amplified by hearing aids or cochlear implants. **Methods.** The sample consisted of 55 children aged 3–6 years, diagnosed with prelingual bilateral hearing impairment with a hearing threshold above 90 dB. All examined children had average intellectual abilities and no additonal impairments. The sample was divided into 2 groups: E1 group consisted of 30 children with cochlear implants and E2 group consisted of 25 children who were amplified by individual hearing aids. Research methodology included a Test of Vocabulary. The testing was performed individually. A year after the testing, a retest was done. Statistical analysis was performed using the SPSS v. 17 for Windows. **Results.** The largest number of children had average achievements on a Test of Vocabulary during initial testing. After a year (retest) significant improvements were noticed. A large number of children had above average achievements (46.7% in the E1 and 36% in the E2 group) while the number of children with below average achievements was significantly reduced (3.3% in the E1 and 8% in the E2). A comparative analysis of the test and those with gearing aids achievements showed that there was no statistically significant difference between children with cochlear implants and retest. **Conclusion.** Significant improvement of the achievements on retest in both groups can be explained by positive effects of systematic, planned, intensive and continuous rehabilitation of hearing impaired children, and not by application of certain type of hearing amplification.

Key words:
hearing disorders; child, preschool; hearing aids; cochlear implants; speech; rehabilitation; vocabulary; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Početna iskustva sa rehabilitacijom dece sa kohlearnim implantima i česte debate u vezi efekata njihove primene nameću neophodnost poređenja efekata rehabilitacije govora dece sa slušnim pomagalima sa decom koja imaju kohlearne implante. Cilj ovog rada bio je da se proceni i uporedi nivo razvijenosti leksičkog fonda dece oštećenog sluha koja su uključena u proces rehabilitacije slušanja i govora, a koja su amplifikovana slušnim aparatima ili kohlearnim implantom. **Metode.** Uzorak je obuhvatio 55-oro dece uzrasta 3–6 godina kod kojih je dijagnostifikovano prelingvalno obostrano oštećenje sluha sa pragom čujnosti od preko 90 dB. Sva deca, su imala prosečne intelektualne sposobnosti i bila su bez dodatnih oštećenja. Uzorak je podeljen na grupu dece koja su kohlearno implantirana (E1 grupa = 30) i grupu dece koja su amplifikovana individualnim slušnim aparatima (E2 grupa = 25). U istraživanju je korišćen Test rečnik. Testiranje je obavljano individualno. Nakon godinu dana od testiranja, rađen je retest. Statistička ob-

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rada rezultata izvršena je pomoću je programom SPSS v. 17 for WIDOWS. **Rezultati**. Najveći broj ispitanika pokazao je prosečna postignuća na Test rečniku, na prvom testiranju. Nakon godinu dana (na retestu) uočena su značajna poboljšanja. Veliki broj ispitanika imao je iznadprosečna postignuća (46,7% u grupi E1 i 36% u grupi E2), a smanjen je i broj ispitanika koji su imali ispodprosečna postignuća (3.3% u grupi E1 i 8% u grupi E2). Komparativnom analizom postignuća dece (na testu i retestu) sa kohlearnim implantom i dece sa individualnim slušnim aparatima primećeno je da između pomenutih grupa ispitanika nije bilo statistički značajne razlike. **Zaključak.** Na osnovu dobijenih rezultata istraživanja možemo zaključiti da je značajno poboljšanje postignuća ispitanika na retestu u obe ispitivane grupe moguće objasniti pozitivnim efektima sistematski planirane, intenzivne i kontinuirane rehabilitacije dece oštećenog sluha, a ne primenom određenog tipa slušne amplifikacije.

Ključne reči:
sluh, poremećaji; deca, predškolska; sluh, pomagala; kohlea, implantat; govor; rehabilitacija; rečnici; ankete i upitnici.

Introduction

Verbal communication is a basic form of communication among people. The ability to speak enables a human being to "liberate" the mind and make it available to others. Speech nuclei as a specific human characteristics are presented at birth in the first newborn cry [1]. From that moment, speech and language development are used through established interconnected and conditioned stages [2].

Speech and language difficulties are reflected in the overall development of a child's personality. For proper speech and language development, among many other factors, preserved auditory perception is necessary [3]. Poor auditory perception compromises spontaneous speech and language development and leads to a smaller or larger delays in its development [4].

Hearing impairment researches (which include studying deafness and hearing loss in all aspects of a child's psychophysiological development as well as researches in otorhinolaryngology) in the last decade was characterized by audiology technology advancement and more expansive approach to cochlear implantation in children with severe hearing impairment as an alternative to hearing aids.

Initial experiences in rehabilitation of children with cochlear implants and frequent debates regarding the effects of their application have imposed the necessity to compare the effects of speech rehabilitation of children having hearing aids with those having cochlear implants.

In practice, such findings can contribute to further evaluation of quality and they can facilitate diagnostic choice of a certain hearing amplification type in the process of speech rehabilitation. In the same manner, this research aims to provide additional arguments to avoid simplifications, euphoric fashion or unfounded criticism.

Taking into account the experience and findings gained by researchers [5–11], it may be concluded that the application of a cochlear implant has recently given significant results in improving a hearing status in prelingually deaf children with severe hearing impairment.

Regarding speech and language development, some recent researches have shown that children with cochlear implant use age-appropriate learning strategies. They also have appropriate level of expressive vocabulary and semantic feature knowledge like children with normal hearing. In general, their cognitive capacities are adequate in managing the knowledge of words and their usage and they are the same as in children with normal hearing [12].

On the other hand, there are some researchers who, although they agree with the fact that the children with implants achieve higher hearing threshold and have better speech and language abilities, hold the opinion that the results are individual and unpredictable [13].

When analyzing the vocabulary, there are researches which confirmed that children with cochlear implants had less vocabulary knowledge than children with normal hearing. This researches also indicated that vocabulary knowledge of children with cochlear implants was highly related to the age of implantation, duration of implantation, chronological age and socioeconomic status [14].

The aim of this study was to determine and compare the level of lexical development of hearing impaired children who are involved in a systematic, continuous and intensive process of hearing and speech and language rehabilitation and who are amplified by hearing aids or cochlear implants.

Methods

The research was conducted in 2008 and 2009 at the Department of Hearing and Speech Rehabilitation at the Institute for Otolaryngology and Maxillofacial Surgery of the Clinical Center of Serbia and at the "Children's Home" of the University Medical Center Zvezdara, Center for Persons with Hearing Impairments in Banja Luka and Institute for Psycho-physiological and Speech Disorders "Dr Cvetko Brajović" in Belgrade. The subjects were tested on two occasions – at the time when they were given consent for cochlear implants (test) and a year after the implementation (retest).

The sample included 55 pre-school respondents aged 3–6 years. All the children from the sample had mutual prelingual hearing impairment with the hearing threshold over 90 dB; they had average intelligence. No children had any additional impairment. In the period prior to obtaining consent for the cochlear implant, all examined children had been involved in intensive rehabilitation treatment which included the stimulation of hearing, speech and language development as well as the integration into the social environment. The treatment was carried out according to a plan and program which is a standard for the appropriate age, the type and degree of hearing impairment as well as the current level of speech and language development. In 30 children who were

tested, cochlear implant was placed (E1 group), while 25 patients continued to use individual hearing aids after receiving the consent for cochlear implant (E2 group). After our testing, all examined children continued with intensive, systematic and planned hearing and speech rehabilitation. The same principles in the area of vocabulary development were followed during rehabilitation of the examined children regardless the applied type of hearing amplification. First, words were adopted as a global unity after which their analytical shaping and processing of individual sounds were elaborated. The process of words acquisition was realized through the stages of detection, discrimination, identification, memory and functional use. At any time, primary impact was on auditory perception of words while visual perception was secondary. Connection between the auditory perception of words and the level of vocabulary development was strictly respected. After one year, a control test (retest) was conducted.

Test of Vocabulary by Vasić [15] for estimation of lexical range was used for the whole sample. The testing was performed individually. The most of the words in this test were nouns (50%) which were the most frequent part of speech in children's vocabulary. The test consisted of 2 parts. The first part contained specific nouns which could be illustrated visually. The second part of the test included abstract nouns which needed to be tested since they indicated a level of child's language development and not only the richness of the child's vocabulary. The abstract nouns were chosen on the basis on their frequency in an active child's vocabulary. The number of specific nouns decreases as child gets older while the number of abstract nouns increases.

Each subtest regarding vocabulary referred to specific age. The test for the age between 3 and 4 years consisted of 20 nouns; for the age between 4 and 5, it consisted of 40 nouns; for the age of 5–6, another 20 nouns were added. The list of 100 words made the total vocabulary test.

Total marks on the test for 3-year-old children was 20 points; for those aged 4 – it was 40 points, for those aged 5 – it was 60 points, for those aged 6 – it was 80 points and for those aged 7 – it was 100 points. That is, each word from the test brought 1 point.

Based on the obtained testing results, we divided the subjects into 3 groups: unsuccessful, average and above average for the observed age.

The test for the age of 3 (test and retest): 0–4 – unsuccessful for the examined age; 4.5–4 – average for the examined age; >14.5 – above average for the examined age.

The test for the age of 4 (test and retest): < 14 – unsuccessful for the examined age; 14.5–20 – average for the examined age; > 20.5 – above average for the examined age.

The test for the age of 5 (test and retest): < 17 – unsuccessful for the examined age; 17.5–30 – average for the examined age; > 30.5 – above average for the examined age.

The test for the age of 6 (test and retest): < 25 – unsuccessful for the examined age; 25.5–40 – average for the examined age; > 40 – above average for the examined age.

Test for the age of 7 (test and retest): < 26 – unsuccessful for the examined age; 26.5–55 – average for the examined age; > 55.5 – above average for the examined age.

We designed a protocol applied in our research with the aim to collect data about subjects which we thought would be necessary and useful when analysing the testing results. The data were taken from children's medical records and through interviews with their parents. The protocol collected the information regarding the following: sex and age, intellectual capacity, the presence of any additional impairment, type and degree of hearing impairment, the time when the hearing impairment diagnosis was established, amplification time, amplification modality, the age when the cochlear implant was provided, the time when rehabilitation started and the length of rehabilitation process.

The obtained results were analyzed by descriptive statistical methods The analysis of relation between dependent and independent variables was done by mathematical algorithms within the application of the correlation analysis which included defining a vector orientation as well as quality and quantity of relations between the compared variables. Statistical analyses were made in program SPSS v. 17 for Windows.

Results

The tested sample included 30 male and 25 female children with hearing impairment. In the E1 group there were 17 boys and 13 girls while in the E2 group there were 13 boys and 12 girls (Table 1).

Possible influence of time when diagnosis of hearing impairment was given on speech and language development as well as on the vocabulary richness is presented in Table 1.

The amplification time and the time of rehabilitation treatment commencement are significant for the level of developing lexical range in hearing impaired children. The largest number of children from the whole tested sample was amplified at the age of 19–30 months (30 children), while basically the same number of children was amplified at the age to 18 months (13 children) and after 30 months (12 children). When compared the E1 and E2 group, it may be noticed that the same number of children was amplified at the age of 19-30 months (15 children). In the E1 group 11 children were amplified at the age up to 18 months and 4 children at the age after 30 months, while in the E2 group only 2 children were amplified at the age up to 18 months and 8 children at the age after 30 months (Table 1).

The analysis of the time when the rehabilitation started showed that the largest number of children from the whole sample (27) started the rehabilitation at the age of 19–30 months.

The length of rehabilitation is also a very significant factor which influences the richness and quality of vocabulary in hearing impaired children. The length of rehabilitation of children according to mode of amplifiers is presented in Table 1.

Considering the children's age when the cochlear implant was placed, it is necessary to point out that only 4 children got the implant at the age of 2 years. Most of the children (20 children) were implanted at the age of 2-4; 6 children got the implant at the age over 4 (Table 1).

Table 1

Individual and functional characteristics of children according to mode of amplifiers

Parameters	E2 (n = 25) n (%)	E1 (n = 30) n (%)	Total n (%)
Gender,			
male	13 (23.6)	17 (30.9)	30 (54.6)
female	12 (21.8)	13 (23.6)	25 (45.4)
The age when hearing impairment was diagnosed (months)			
< 18	11 (20)	15 (27.3)	26 (47.3)
19–30	8 (14.5)	11 (20)	19 (34.5)
> 30	6 (10.9)	4 (7.3)	10 (18.2)
The age when child was amplified (months)			
< 18	2 (3.6)	11 (20)	13 (23.6)
19–30	15 (27.3)	15 (27.3)	30 (54.6)
> 30	8 (14.5)	4 (7.3)	12 (21.8)
The age when child started with rehabilitation in (months)			
< 18	4 (7.3)	11 (20)	15 (27.3)
19–30	12 (21.8)	15 (27.3)	27 (49.1)
> 30	9 (16.3)	4 (7.3)	13 (23.6)
Duration of rehabiliatation (years)			
< 3	2 (3.6)	5 (9.1)	7 (12.7)
3–5	19 (34.5)	18 (32.7)	37 (67.3)
> 5	4 (7.3)	7 (12.7)	11 (20)
The age when cochlear implant was done (years)			
< 2		4 (7.3)	4 (7.3)
2–4		20 (36.4)	20 (36.4)
> 4		6 (10.9)	6 (10.9)

E1 – group of children with cochlear implants; E2 – group of children amplified by individual hearing aids.

Before the analysis of Vocabulary Test results was made, we also analyzed the age of children during the first testing as well as during retesting (Table 2).

By using the Test of Vocabulary in our research, we had the aim to determine to what extent the children with hearing impairment developed their lexical knowledge, that is, to what extent they passed from the passive to active vocabulary phase. The testing results were presented in Table 2.

Results in Table 2 indicated that, at the Vocabular Test and retest, the children with cochlear implant achieved approximately the same results as the children with hearing aids.

The test results in both examined groups showed that the majority of children achieved the average results, a large number of them achieved results which were below standards, while fewer of them achieved results above the group average.

Retest results showed improvement in lexical range in both tested groups. Children with cochlear implants had better results than children with hearing aids, but there was no statistically significant difference.

Average scores were the most frequent in both groups. However, the number of children with above-average achievements was higher while the number of children with below average achievements was reduced. Since the results in both groups followed the same trends, statistically significant difference was not observed ($p = 0.672$).

Table 2

Children' achievements at the Test of Vocabulary regarding the type of hearing amplification in the E1 and E2 group (number of children on defined examined level)

Parameter	Test, n (%)		Retest, n (%)	
	E2	E1	E2	E1
Age at first testing in years				
3–4	7 (28)	5 (17)	1 (4)	1 (4)
4–6	13 (52)	19 (63)	16 (64)	13 (43)
> 6	5 (20)	6 (20)	8 (32)	16 (53)
total	25 (100)	30 (100)	25 (100)	30 (100)
Achievements for examined age				
below average	8 (32)	11 (36.7)	2 (8)	1 (3.3)
average	11 (44)	13 (43.3)	14 (56)	15 (50)
above average	6 (24)	6 (20)	9 (36)	14 (46.7)
Total	25 (100)	30 (100)	25 (100)	30 (100)

E1 – group of children with cochlear implants; E2 – group of children amlified by individual hearing aids.

Discussion

Test results on the Test of Vocabulary showed that the largest number of children had average lexical knowledge. Somewhat smaller number of tested children had lexical range below standards, and the smallest number of children had lexical range above-average. Such results are the consequence of the Test of Vocabulary unconformity while applying to hearing impaired children (the test was standardized for the typical population of children). The criteria imposed by this test are too high for hearing impaired children, and, therefore, we were forced to classify the results achieved by subjects on this test according to maximum achievements of subjects from the specific group.

The aim of the retest was to determine any potential changes in the quality of developed lexical range, compared to the first testing, along with continuous and systematically planned and performed rehabilitation, and with applying a certain type of hearing amplification.

Retest results showed, like the ones from the test, that the majority of children had the average lexical range. However, there were significant changes in the groups of children with above-average and lexical range developed below average. The number of participants in the below significantly decreased (from 19 subjects on the Test to 3 subjects on the retest), while the number of subjects with lexical range above-average increased almost twice (12 subjects on the Test and 23 on the retest). Undoubtedly, a high-quality change happened to all children from our sample in development of the lexical knowledge. The obtained retest results indicated positive effects of systematically performed speech and language treatment on development of the lexical range in children with hearing impairment, no matter whether they had cochlear implant or individual hearing aid. These findings are in relation to the literature which confirmed that children with cochlear implant could benefit from the treatment focused specifically on learning language structures, despite their phonological deficits as a consequence of reduced auditory perception [16]. Other research pointed to a fact that acquiring spoken language was facilitated by good audibility which was provided by a cochlear implant as well as with memory abilities and phonological learning [17]. On the other hand, many researches dealing with language development in children with cochlear implants suggest that there was a good reason to suspect that even the most successful children with cochlear implants go through different lexical processes and representations than children with normal hearing, particularly in case of phonological representation and processing [18]. Our explanation would be based on the fact that success in lexical range development lies in an intensive speech and language treatment of hearing impaired children with cochlear implants or hearing aid. This systematically planned and performed speech and language treatment may provide children with hearing amplification of the same lexical processes that children with normal hearing get through.

An explanation about vocabulary acquisition given by Storkel [19] may also be applied to the analysis of lexical development in hearing impaired children. This explanation points to at least 2 neurocognitive processes which are the base for learning words: learning from an input during training and memory evolution during periods between training sessions. Word acquisition by healthy adults consists of learning from an input which is swift and stable, whereas memory evolution may be vulnerable on the pathway to mastery. That means that success in learning from the input is linked to positive outcomes from memory evolution. Similar principle for learning words by hearing impaired children can be applied to intensive speech and language treatment, regardless the fact that these children have cochlear implants or hearing aids.

Comparison between testing and retesting results in children with cochlear implants and children amplified with individual hearing aids indicated that there was not a statistically significant difference between these two groups. The most of the children from both tested groups had average achievements on initial testing by the Test of Vocabulary. An improvement in development of lexical range was registered in both tested groups by applying retesting (an increased number of subjects with above-average results and decreased number of subjects with below average results). Children with cochlear implants showed small advantage over those ones with hearing aids, but that difference was not statistically significant. Therefore, achievements of children with cochlear implants and those with hearing aids were almost identical in the domain of active vocabulary development.

According to Ostojić's [20] exploration of Elber's views, children with cochlear implants made a progress in a similar manner to children with hearing aids. Regardless the time and effort made at different levels, auditory stimulation usually must be adjusted to a child' learning pace. One of the basic requirements in working with children is to respect the developmental phases. If any of the developmental phases is left out or late, a child needs to be stimulated and to be given time to adjust; later on, we should expect the learner's active participation in the next phase. This model is most often applied in rehabilitation of children with cochlear implants [20].

The mentioned views explain the absence of statistically significant difference in comparing results at the Test of Vocabulary of children with different types of hearing amplification due to equal absence or delay during developmental phases which caused equally good or bad results on the test. On the other hand, these results should serve as a guide in practical work on speech-language rehabilitation of these children, inhibiting the expectations of experts and parents to those elements in speech and language development of deaf children at whom the efficiency of cochlear implant is reasonably exaggerated in comparison to hearing aids (quality of the basic laryngeal voice and voice articulation).

Our research results definitely point to positive effects which intensive and continuous rehabilitation had on the development of lexical range in hearing impaired children. Generally speaking, the intensive rehabilitation provided significant improvement in children's lexical knowledge, but the efficiency of rehabilitation was also noticed in overcoming the obstacles that hearing impaired children have during speech-language developmental stages. This is the fact we should not neglect. It confirms the necessity of expert and

professional approach to early detection, diagnostics and rehabilitation of hearing impaired children.

The limitation of this study refers to the small number of respondents. Future studies which would include larger sample of hearing impaired children may demonstrate more clearly whether the model of auditory amplification has the positive impact on speech and language therapy or the intensive speech and language treatment has the major role in speech and language development of hearing impaired children.

Conclusion

Cochlear implant itself or applications of individual hearing aids do not guarantee successful rehabilitation without quality speech and language therapy. Technical aids, no matter the level of their technological perfection, are just aids. In certain segments, they can improve the rehabilitation quality, but speech and language therapist with his/her expert knowledge, experience, good intentions and humane approach is of key importance. The best guarantee for speech and language development which also include lexical development in hearing impaired children is a good combination of a human factor in the form of (a speech and language therapist) and technical means and aids which we use in the process of speech-language rehabilitation.

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The impact of somatic symptoms on depressive and anxiety symptoms among university students in central Serbia

Uticaj somatskih tegoba na nastanak depresivnosti i anksioznosti kod studenata u Srbiji

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Abstract

Background/Aim. Depression and anxiety problems are a major public health concern due to their high prevalence rates, difficult treatment, and often chronic course**.** This study examined the impact of somatic symptoms on depressive and anxiety symptoms among university students in Serbia. **Methods**. A cross-sectional study was performed among 1,940 students using a questionnaire specially designed for this study which included presence of Somatic and Non-specific Mental Symptoms (SNMS), Beck Depression Inventory and Beck Anxiety Inventory. The presence of somatic and associated non-specific mental symptoms over the last six months served as the basis for creating a new variable called SNMS score. **Results.** Receiver operating characteristic (ROC) curve showed that the SNMS score might be a very good marker for the distinction of students with or without depressive symptoms (area = 0.754, $p < 0.05$). The threshold value was 8.50 (sensitivity 67.6%, specificity 69.4%). Binary logistic regression showed that Odds ratio was 1.052 [95% confidence interval (CI) 1.045–1.059], which means that an increase in the value of the SNMS score by 1 increases the risk of depressive symptoms by 5.2%. ROC curve showed that the SNMS score might be an excellent marker for the distinction of students with or without anxiety symptoms (area = 0.800, $p < 0.05$). Limit value (cut-off) was 7.50 (sensitivity 74.2%, specificity 71.6%). Binary logistic regression showed that odds ratio was 1.056 (95% CI 1.049–1.064), which means that increasing the value of SNMS score by 1 increases the risk of anxiety symptoms by 5.6%. **Conclusion.** The SNMS score might be a state marker for the screening and distinction of students with depressive symptoms, and excellent state marker for screening and making distinction between students with anxiety symptoms and the students who do not have these symptoms.

Key words:
depression; anxiety; signs and symptoms; psychophysiologic disorders; surveys and questionnaires; students; serbia.

Apstrakt

Uvod/Cilj. Depresija i anksioznost predstvljaju ozbiljan javnozdravstveni problem koji karakteriše visoka prevalencija, hronični tok i dugotrajan proces lečenja. Cilj istraživanja bio je da se utvrditi uticaj somatskih i prodruženih nespecifičnih psihičkih simptoma na nastanak depresivnosti i anksioznosti kod ispitivane studentske populacije. **Metode.** Istraživanje je sprovedeno kao epidemiološka studija preseka na uzorku od 1 940 studenata, korišćenjem upitnika konstruisanog za potrebe istraživanja koji je pored karakteristika somatskih i pridruženih nespecifičnih psihičkih simptoma (*Sometic and Non-specific Mental Symptoms* – SNMS), obuhvatao Bekovu skalu za procenu depresivnosti i Bekovu skalu za procenu anksioznosti. Prisustvo određenih somatskih i pridruženih nespecifičnih psihičkih simptoma tokom poslednjih šest meseci poslužilo je za kreiranje nove varijable nazvane SNMS skor. **Rezultati.** *Receiver operating characteristic* (ROC) kriva pokazala da je vrednost SNMS skora vrlo dobar marker za distinkciju studenata sa depresivnošću od studenata bez nje (area = 0,754, $p < 0,05$). Granična vrednost bila je 8,50, senzitivnost 67,6%, a specifičnost 69,4%. Binarna logistička regresija pokazala je da vrednost SNMS utiče na pojavu depresivnosti. Količnik rizika (*Odds ratio* – OR) bio je 1,052 [95% interval poverenja (IP) 1,045–1,059], što znači da se povećanjem vrednosti ove promenljive za 1, povećava rizik od nastanka depresivnosti za 5,2%. ROC kriva pokazala je da je vrednost SNMS odličan marker za distinkciju stu-

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denata sa anksioznošću, od studenata bez anksioznosti (area = 0,800, $p < 0,05$). Granična vrednost (*cut-off*) bila je 7,50, pri čemu je senzitivnost iznosila 74,2%, a specifičnost 71,6%. Binarna logistička regresija pokazala je da vrednost SNMS utiče na pojavu anksioznosti. OR bila je 1,056 (95% IP 1,049–1,064), što znači da se povećanjem vrednosti ove promenljive za 1, povećava rizik od anksioznosti za 5,6%. **Zaključak.** Vrednost SNMS skora može biti dobar marker za skrining i distinkciju studenata sa depresivnošću od studenata bez nje, i odličan marker za skrining i distinkciju studenata sa anksioznošću od studenata bez anksioznih simptoma.

Ključne reči:
depresija; anksioznost; znaci i simptomi; psihofiziološki poremećaji; ankete i upitnici; studenti; srbija.

Introduction

Mental health problems are a serious public health problem concern due to their high prevalence rates, difficulties during the treatment, chronic course [1] and at the same time a source of immense human suffering [2].

Mental disorders account for a large proportion of the disease burden in young people and many such illnesses have typically their first onset during the university age [3, 4].

Lack of recognition of mental health problems among young people, becomes important challenge for medical professionals in the struggle for the disorder identification and consequent treatment, as early as possible [5].

Depression and anxiety increasingly emphasize the importance of somatic, comorbid symptoms, which increase both medical and economic burden to the society [6]. For example, shoulder or arm pain/discomfort is common among college students. Researchers reported that Nigerian undergraduates had a high prevalence of musculoskeletal pain, where shoulder pain was most common. In Australia, musculoskeletal disorders were a widespread problem for university students; and in the United States, college students reported low back pain, and the majority had musculoskeletal discomfort during/after computer use. Likewise, in Saudi Arabia, about the fourth to the fifth of students complained of headache (17%) and fatigue (24%) [7]. It is well documented that pain or aches in shoulders, arms and neck are prevalent in many societies and are an economic problem due to sickness absence and health-care costs [8].

Several studies of the relationship among anxiety, depression, and somatic symptoms which included general population have already been conducted in many countries. Based on such experiences, three explanatory hypotheses about the nature of the relationships can be offered. Firstly, depression and anxiety disorders may influence the onset of physical symptoms in many ways [9] e.g. altered perception of physical sensations [10]. Secondly, somatic symptoms, or different types of physical limitations may be predictors of onset of depressive and anxiety disorders. Finally, according to the third hypothesis, numerous environmental, biological and psychological factors may independently influence the onset of both mental disorders and somatic complaints [11].

In clinical practice, there is often so-called masked depression. It is characterized by somatic "mask", which is comprised of a wide range of somatic disorders. It is not rare that people suffering from masked depression seek help from doctors of different specialties, change healthcare facilities and most often they are diagnosed with hypochondria. In our culture, this projection of psychological problems to somatic symptoms is explained by, among other things, greater social acceptability of somatic disorders. Stigma and negative attitudes toward seeking help from a psychiatrist are still barriers to improving mental health [11].

It is estimated that 7.3% to 11% of all patients suffering from depression suffer from masked depression, and on average, an interval between first signs of illness and referral to a psychiatrist, is 3–5 years. The most common motives for the referral are negative findings of somatic examinations and lack of therapy response to the applied somatic therapy. Patients who suffer from this disorder usually complain about headaches in the form of twinges, pinching, pain in the face and teeth and often closely resemble migraine. In addition, there are pains in the lumbar spine and surrounding spinal muscles, and the symptoms are often interpreted as spondyloarthrosis or discopathy. The occurrence of paresthesia in the region of extremities, the feeling of heaviness of extremities "as if they were filled with lead" are common; there is also the phenomenon of restless legs, shortness of breath and pain in various parts of the body. There are also vegetative symptoms which frequently appear as dizziness, feeling of emptiness in the head, chest pressure, pain in the heart, tachycardia, dryness in the mouth, anuria, dysphagia, meteorism or difficult breathing. Therefore, the masked depression is a disorder that can mimic different somatic diseases making its diagnosis and treatment difficult [12].

The aim of this study was to examine the impact of somatic symptoms on depression and anxiety symptoms among a group of University students in Central Serbia.

Methods

Study design and participants

The research we have conducted was a cross-sectional survey of students attending University of Kragujevac, Serbia, during the 3-years period, that is, from 2012 to 2015. University of Kragujevac, with its twelve faculties, is a state-owned university in central Serbia. Six of its faculties are located in Kragujevac while others are located in five towns in central Serbia, covering the area with more than 2,500,000 inhabitants. All faculties were selected for the survey: Faculty of Agronomy, Faculty of Economics, Faculty of Engineering, Faculty of Mechanical and Civil Engineering, Faculty of Medical Sciences, Faculty of Education, Faculty of Law, Faculty of Natural Sciences and Mathematics, Faculty of Technical Sciences, Teachers Training Faculty, Faculty of

Philology and Arts, Faculty of Hotel Management and Tourism. The students were randomly sampled from each study year from each faculty, in proportion to the size of the faculty in relation to the total number of students at the University. The sample for this survey, was randomly selected: 10.70% students, out of the total of 18,123 students. The students were sorted out from the University student database according to previously generated random order (random computer function).

Procedure

A self-administered anonymous questionnaire was used and it comprised Somatic and associated Nonspecific Mental Symptoms (SNMS), Beck Depression Inventory and Beck Anxiety Inventory (BAI). Ethical approval was obtained from the Faculty of Medical Sciences Ethical Committee. Participation was voluntary with no financial or other motivation. Informed consent was obtained and confidentiality of the responses was assured. The study was conducted in the participants' classrooms by the leading researcher (I.V.S). Those who were absent during the distribution of questionnaires were excluded. The research was completed within 2 years.

Instrument

A self-assessment questionnaire (which was assembled for this study) with detailed subdomain questions was used to determine SNMS. Symptoms of depression were evaluated through the Back Depression Inventory – revision of original instrument (BDI-IA) scale. This scale was developed in the 1960's and is one of the most widely used instruments for measuring the severity of depression, with the focus on behavioral and cognitive aspects of the disorder. It was designed to document a variety of depressive symptoms which the individual experienced over the preceding week. It consistes of 21 items, each answer being scored on a scale ranging from 0 to 3 [12].

Symptoms of anxiety were evaluated through the Back Anxiety Inventory (BAI) scale, a short list describing 21 anxiety symptoms which bothered them in the previous week. The scale consistes of 21 items, each answer being scored on a scale ranging from 0 to 3 [13].

The presence of SNMS over the last six months served as the basis for creating a new variable called SNMS score. Questions from the questionnaire, that were related to the presence of specified nine symptoms reported over the last six months (headache, abdominal pain, back pain, fatigue, despondency, irritability, nervousness, sleep problems, dizziness), were rated with five-graded responses (rarely or never – 0, almost every month – 1, almost every week – 2, more than once a week – 3, almost every day – 4). Therefore, the maximum score for these symptoms was 36. Some clinical findings suggest that somatic and associated nonspecific mental symptoms like despondency, irritability, nervousness, sleep problems may dominate the clinical picture and develop prior to other symptoms and signs of depression [14].

Statistical analysis and assessment

Data analysis was carried out using IBM SPSS (Statistical Package for the Social Sciences) software version 19.0. The qualitative variables were presented in number and percentage. The continuous variables (depression, anxiety and symptoms scores), were presented as means and standard deviation (SD). Descriptive statistics for somatic symptoms, depressive and anxiety symptoms were calculated, expressed as appropriately in frequencies, mean values and standard deviation. Student's *t*-test, Fisher's exact test, χ^2 test, were used to look for any existing differences between somatic symptoms, and depressive/ anxiety symptoms. All tests were 2-tailed, and the level of significance was set at $p \leq 0.05$. Binary logistic regression analysis was used to determine associations between depressive, anxiety symptoms and somatic symptoms. The results were reported as odds ratios (OR) with 95% confidence intervals (CI).

Results

Out of 1,968 distributed questionnaires, a total of 1,940 (98.6%) students completed the questionnaire among which there were 34.7% of males and 65.3% of females) the mean age of the participating students was 21.04 (SD = ± 2.23) years with the range of 18–57 years.

Distribution of somatic and associated nonspecific mental symptoms of the sample are summarized in Table 1.

Table 1

Distribution of somatic and associated non-specific mental symptoms in the student population

Simptoms	Rarely or never (%)	Almost every month (%)	Almost every week (%)	More than once a week (%)	Almost every day (%)
Dizziness	84.7	7.7	3.6	2.4	1.5
Problems with sleep	58.0	16.5	8.7	9.0	7.8
Nervousness	22.4	30.9	19.3	15.5	11.9
Irritability	48.2	27.9	11.6	7.9	4.4
Despondency	59.9	20.6	8.8	6.4	4.3
Back pain	52.8	21.1	10.5	8.4	7.2
Fatigue	57.6	19.5	9.7	8.0	5.2
Abdominal pain	45.6	38.6	7.7	6.5	1.6
Headache	41.2	22.3	14.9	14.2	7.4

***symptoms students felt in the lost six months before the study begining.**

The difference between the mean values of SNMS score between students with depressive symptoms and students without depressive symptoms was statistically significant ($p < 0.05$). The mean value of the SNMS score of students without depressive symptoms was 5.00 (range, 3.00 to 10.00), while the mean value of the new variable in students with depressive symptoms was 12.00 (range, 7.00 to 18.00).

Receiver operating characteristic (ROC) curve showed that the SNMS score might be a very good state marker for the distinction of students with depressive symptoms from students without it (area = 0.754, $p < 0.05$). The threshold value was 8.50, the sensitivity was 67.6% and specificity 69.4% (Figure 1).

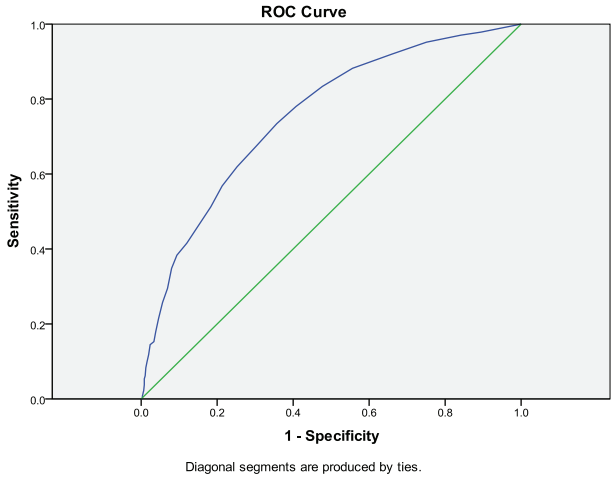

Fig. 1 – Receives operating characteristic (ROC) curve of the sum score of Somatic and associated Non-specific Mental Symptoms according to depressive symptoms.

Binary logistic regression showed that SNMS score might affect the occurrence of depressive symptoms. Odds ratio was 1.052 (95% CI 1.045 to 1.059), which means that an increase in the value of the SNMS score by 1 increases the risk of depressive symptoms by 5.2% over the baseline population rate.

The difference of mean values of the SNMS score between patients with anxiety symptoms and those without it was statistically significant, too ($p < 0.05$). The mean value of the SNMS score of the somatic and associated nonspecific mental symptoms in students without anxiety symptoms amounted to 4.00 (range, 2.00 to 8.00), whereas the mean value of the new variable in students with anxiety symptoms was 12.00 (range, 7.00 to 17.00).

ROC curve shows that the SNMS score might be valuable state marker for the distinction of students with anxiety symptoms, from students without anxiety symptoms (area = 0.800, $p < 0.05$). Limit value (cut-off) was 7.50, wherein sensitivity was 74.2% and specificity was 71.6% (Figure 2). Binary logistic regression showed that the SNMS score might affects the occurrence of anxiety symptoms. Odds ratio was 1.056 (95% CI 1.049 to 1.064), which means that an increase in the value of score of somatic symptoms by 1 increases the risk of anxiety symptoms by 5.6%.

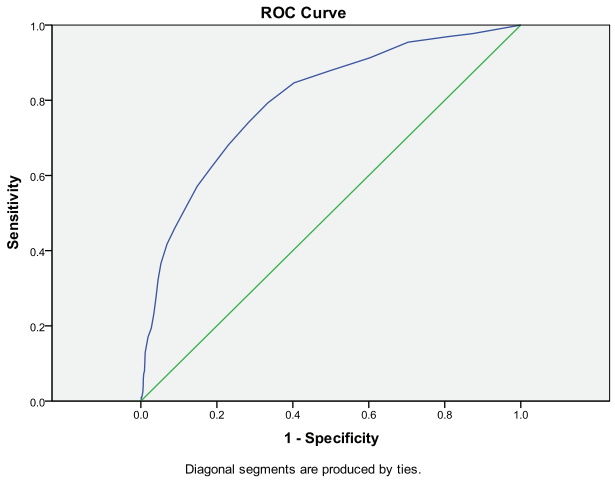

Fig. 2 – Receives operating characteristic (ROC) curve of the sum score of Somatic and associated Non-specific Mental Symptoms according to anxiety symptoms.

Discussion

Our study is one of the largest epidemiological studies regarding mental health status and somatic symptoms among university students in this region. The first objective of this study was to examine the distribution of same somatic symptoms among university students in central Serbia.

Our results show that headache and back pain represent common symptoms among university students, i.e. 22.3% of respondents had headache almost every month and 7.4% on daily basis over the last six months. The literature indicates that most common somatic symptoms among student population are headache, menstrual cramps, back pain, fatigue and sleeping problems [15]. Worldwide, 54.4 % of the adolescent population suffer from headache. A study conducted in Germany shown that over 80% of adolescents had headache symptoms in the last 6 months. One study showed that 30.3% of Chinese adolescents experienced headache more than once a week during the previous 3 months [16]. The data combined from 56 independent studies (analyzing a total number of 34,904 students) revealed that pooled migraine prevalence amounts to 16.1% [17]. The study conducted on a sample of 4,406 students in China showed that the prevalence of migraine among university students was 9.0% [18], which was in accordance with the prevalence found in Chinese adults in the general population (8,9%) [19]. Previous epidemiological studies linked anxiety and depression with migraine suggesting the presence of such comorbidity among 30%–84% people. It was proven that depression and anxiety increase the risk of migraine when they occur separately as well, however, if the depression and anxiety occur together, the risk becomes even higher [20].

The results of this study suggest that 21.1% of students had back pain during the last six months almost every month, while 7.2% had back pain every day. The obtained results reveal a high prevalence of back pain among student population. The data from the current literature showed that back pain in student population varies between 20%–70%. In several studies, obtained values for back pain prevalence were

higher than the results obtained by this study. For example, in a study conducted in China on the sample of 2,849 adolescents, the prevalence amounts to 41.4%, among Brazilian school children to 55.5% (n = 802) and 51.3% among 546 Danish adolescents [21.] The reason may be that heavy academic pressure induces an increase in risk factors for somatic symptoms [elevated levels of mental stress, lack of sleep and changes in adolescent living habits (smoking, alcohol and coffee consumption)] which can together influence the occurrence of mental disorders. Relationship between chronic pain and depression or anxiety attract particular attention in the field. About 35% of patients with chronic pain meet the criteria for an anxiety disorder, while 30% –60% of patients with chronic pain suffer from depression [22]. It was shown that chronic pain in childhood and adolescence heightens the risk of depressive and anxiety disorders in adulthood in specific samples [23].

The main objective of this study was to examine the impact of somatic symptoms on depressive and anxiety symptoms among University students in central Serbia.

In our study, the mean value of the sum score of somatic and associated non-specific mental symptoms among student population without depressive symptoms was 5.00 (range, 3.00 to 10.00), while the mean value of the new variable in students with depressive symptoms was 12.00 (range, 7.00 to 18.00). The mean value of the sum score of the somatic and associated non-specific mental symptoms in students without anxiety symptoms amounts to 4.00 (range, 2.00 to 8.00), whereas the mean value of the new variable in students with anxiety symptoms is 12.00 (range, 7.00 to 17.00).

Our findings are similar to those of the study conducted at two universities in Mexico, which included 506 psychology showing students that the intensity of physical manifestations is more positively correlated with scores derived from two rating scale (Beck Depression Inventory and Social Anxiety Scale for Adolescents) [15]. The indicated that pain-related complaints presented to general practitioners including undefined somatic symptoms (muscle pain, headache, and abdominal pain) are from 2.5 to 10 times more frequent in people with panic disorder, generalized anxiety disorder and major depression than in general population [15]. Patients who felt some kind of somatic pain also had worse mental functioning and higher scores on rating scales for depression, social anxiety and post-traumatic stress disorder [24]. It was suggested in current literature that somatic symptoms directly relate to difficulties in everyday functioning, disability, increased use of psychoactive substances, impaired quality of life as well as reduced use of health care services [6]. The research in the United States showed that 87% of students were feeling mild fatigue while studying. Students who were feeling moderate and severe fatigue scored higher on the BDI scale for the assessment of depression than those who were feeling mild fatigue. Students with severe fatigue also got higher scores on the scale for anxiety assessment [25]. Other studies also reported that depression disorders were in a stronger correlation with somatic problems of the musculoskeletal system [26] while cardiopulmonary symptoms were more associated with anxiety disorders [9, 27].

Our results suggest that the SNMS score might be a very good marker for the distinction of students with depressive symptoms from students without it. In other words, the sum score of somatic and associated non-specific mental symptoms might affect the occurrence of depressive symptoms, which means that an increase in the value of the sum score of somatic and associated non-specific mental symptoms by 1, increases the risk of depressive symptoms by 5.2% over baseline population rate. Several studies have reported the association between depression and pain (e.g. severity, frequency, duration and number of symptoms). Patients with some pain symptoms (e.g. back pain, headache, abdominal pain, chest pain and facial pain) are 3 to 5 times more likely to be depressed than patients without pain, and pain symptoms are associated with at least twice increased risk for coexisting depression. Additionally, one population-based study revealed that subjects with chronic pain (defined as pain felt during most days in at least a month) were 3 times more likely to meet depression criteria than those without chronic pain. The association between depression and pain became stronger as severity of either condition increases. Additionally, more frequent pain episodes and longer duration of pain were associated with depression. An international study showed that patients with pain lasting longer than 6 months were more than four times likely to have a depression disorder than those without chronic pain [28].

Chronic pain and depression are considered to be the harshest expressions of human suffering. Clinical experience suggests that physical pain and depression are often intertwined, and that they are in mutual interaction [22]. Some consider the pain one of the symptoms of depression, which could explain the effectiveness of antidepressants in the suppression of painful condition such as neuropathic pain [29].

Our results indicate that the sum score of somatic symptoms might be a valuable marker for the distinction of students with anxiety symptoms, i.e. the sum score of somatic symptoms might affect the occurrence of anxiety symptoms which means that an increase in the value of the score of somatic symptoms by 1 increases the risk of anxiety by 5.6%.

Data from other studies suggest that anxiety disorders may be present in up to 60% of patients with chronic pain, i.e. more severe chronic pain was associated with more severe anxiety symptoms [30]. Anxiety is associated with pain, as a symptom, regardless of its anatomic localization [19]. The research, conducted by World Health Organization (WHO), which involved 5,447 students in 15 research centers, located in America, Europe, Asia and Africa, found that people with chronic pain were four times more likely to suffer from anxiety disorders and depression [31].

The symptom of chronic pain can be a potential risk factor for recurrent depressive and anxiety disorders. Neuroimaging studies showed overlapping of neural networks of emotions and pain, particularly in prefrontal cortex [32]. Pain could affect regulation of the hypothalamic-pituitary-adrenal axis (increasing cortisol levels) as well as the activity of the autonomic nervous system (increased sympathetic nervous system activity and decreased parasympathetic nervous system activity) which may lead to the onset of new depressive

episodes [33]. On the other side, some studies failed to find association between pain symptoms and recurrence of anxiety [32].

Depressive and anxiety disorders, in comorbidity with physical manifestations, are associated with an increased number of suicides, decreased quality of life and significant economic burden on the health care system [24].

The limitation of our study is the cross-sectional design, which does not permit inferences about possible causal relations between the somatic variables and depressive and anxiety symptoms. It was not possible to assess the test-retest reliability of BDI/BAI in this sample as the survey was anonymous. Another limitation was the self-reported nature of this study. Finally, the sample represents a group of students in just one university of Serbia, which may limit generalizations of the results on the other universities.

Therefore, the careful rater could rather easily screen a student with depression and anxiety with existing and validated tools if additional attention was paid to the domains related to somatic symptoms. Future studies are needed for developing the novel, valid diagnostic rating scales highly specific for detection of depressive and anxiety disorders in students suffering predominantly from non-specific physical complains without obvious causes.

The results of this study illustrated that student population talks about somatic symptoms rather than about mental problems and highlight the importance of recognizing those symptoms and connection between them and depressive and anxiety disorders.

Conclusion

The SNMS score might be a state marker for the screening and distinction of students with depressive symptoms and excellent state marker for screening and making a distinction between the students with anxiety symptoms and the students who do not have these symptoms.

Somatic symptoms could be a predictor of depression and anxiety among students, and early screening could improve their prevention and early diagnose and treatment.

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Conflict of Interest

The authors fully declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Estimation of influenza activity in Vojvodina (Serbia) for five consecutive influenza seasons

Procena aktivnosti influence u Vojvodini (Srbija) tokom pet uzastopnih sezona nadzora nad gripom

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Abstract

Background/Aim. After pandemic 2009/10 influenza season, influenza A (H1N1)pdm09, A(H3N2) and B viruses have continued to circulate in the population. The aim of this study was to describe the epidemiological and virological characteristics of influenza and evaluate values of proposed case definitions of influenza like illness (ILI), severe acute respiratory illness (SARI) and acute respiratory distress syndrome (ARDS) for detecting laboratory-confirmed influenza cases in Vojvodina. **Methods.** We conducted a descriptive epidemiological study using surveillance reports and laboratory data from October 2010 to May 2015 (five surveillance seasons). **Results.** Out of 1,466 samples collected, 720 (49.1%) were laboratory confirmed as influenza. Influenza A infection was more frequently detected than influenza B infection. Using the case definition of ILI was a good predictor for influenza confirmation ($p < 0.05$) during 5 influenza seasons. The predominant age-range of patients with confirmed influenza A (42.2%) and B (43.0%) infections was 30 to 64, but the patients aged from 15 to 29 years were more likely to have influenza A ($p = 0.0168$). In the period from December to January, influenza A (17.8%) was more frequently registered than influenza B (7.6%). The highest number of deaths (19/38) and hospitalized patients (128/402) was registered during the last influenza season (2014/15). The immunosuppressed patients with confirmed influenza infection were more likely to have influenza B than influenza A ($p = 0.0110$). **Conclusion.** Our results indicate that influenza surveillance should be continued and expanded in order to fully assess the burden of the disease in given population.

Key words:
influenza, human; serbia; epidemiology; virology; diagnosis, differential.

Apstrakt

Uvod/Cilj. Nakon pandemijske 2009/10 sezone nadzora nad gripom, virusi influence tipa A (H1N1)pdm09, A(H3N2) i tipa B nastavili su da cirkulišu u populaciji. Cilj ovog istraživanja bio je da se opišu epidemiološke i virusološke karakteristike virusa influence i da se proceni vrednost predloženih definicija slučaja oboljenja sličnih gripu (OSG), teške akutne respiratorne bolesti (TARB) i akutnog respiratornog distres sindroma (ARDS) za otkrivanje laboratorijski potvrđenih slučajeva virusa influence u Vojvodini. **Metode.** Sprovedena je deskriptivna epidemiološka studija upotrebom podataka iz izveštaja u nadzoru i laboratorijskih podataka u periodu oktobar 2010–maj 2015. godine (pet sezona nadzora). **Rezultati.** Od ukupno 1 466 prikupljenih uzoraka, laboratorijska potvrda virusa influence dobijena je kod 720 (49,1%). Infekcija prouzrokovana virusom influence tipa A je češće detektovana u odnosu na onu izazvanu virusom influence tipa B. Korišćene definicije slučaja OSG su bile dobar prediktor za laboratorijsku potvrdu virusa influence ($p < 0,05$) tokom svih pet sezona nadzora. Infekcije virusom gripa tipa A i B najčešće su dokazivane kod bolesnika starosti 30–64 godine (42,2% i 43,0%), a bolesnici starosti 15–29 godina imali su veće šanse da obole od virusa gripa tipa A nego tipa B ($p = 0,0168$). U periodu od decembra do januara virus influence tipa A (17,8%) češće je registrovan nego virus influence tipa B (7,6%). Najveći broj smrtnih slučajeva (19/38) i hospitalizovanih zbog gripa (128/402) registrovan je tokom poslednje sezone nadzora nad gripom (2014/15). Imunokompromitovani bolesnici sa potvrđenim gripom imali su veće šanse da obole od virusa gripa tipa B nego tipa A ($p = 0,0110$). **Zaključak.** Naši rezultati ukazuju na to da je za potpuniju procenu opterećenja populacije virusom influence neophodno nastaviti i proširiti nadzor nad gripom.

Ključne reči:
grip; srbija; epidemiologija; virologija; dijagnoza, diferencijalna.

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Introduction

Influenza is a common seasonal illness causing epidemics worldwide [1]. Influenza surveillance provides information on virus activity and basis for early warning of an upcoming epidemic or pandemic period [2].

Influenza A and influenza B viruses are responsible for recurrent epidemics in humans. Influenza A has more significant impact on public health because of its faster evolution and diverse host range [3].

It is known that influenza A(H1N1)pdm09, A(H3N2) and B viruses continue to circulate in population after the pandemic period. A number of reports from different countries have documented the burden of influenza in the post-pandemic period [4].

Surveillance of influenza at the European level has been conducted since 1996 [5]. According to the model of surveillance conducted in Slovenia [6], sentinel surveillance of influenza like illness (ILI) and acute respiratory infection (ARI) was introduced in the Autonomous Province (AP) of Vojvodina (the northern region of Serbia) in the period 2004/05 (pilot study) and continued for the following 4 influenza seasons. Thanks to the results of this quality surveillance, newly established national influenza surveillance throughout the Republic of Serbia, has been conducted since 2009 [7].

Also, since 2010/11 influenza season along with surveillance of ILI, surveillance of severe acute respiratory infection (SARI) and acute respiratory distress syndrome (ARDS) among hospitalized patients was implemented in Vojvodina [7].

There are very limited data on the contribution of influenza virus to the burden of outpatients and hospitalized patients in Vojvodina. A five-year surveillance study was carried out in Vojvodina to estimate the influenza activity during the post-pandemic period.

Methods

A retrospective study was conducted. We analysed data from Vojvodina surveillance of influenza among outpatients (with ILI) and in hospitalized patients (with SARI and/or ARDS). Surveillance data from the period from October 2010 to May, 2015 were analyzed. We characterized the epidemiology, virology and the predictor values of proposed case definitions to detect influenza virus infection based on our results. The surveillance data were collected for the whole influenza season (from the calendar week 40 of the given year to the calendar week 20 of the following year) [7, 8].

Sentinel surveillance

From 2010 to 2015 seasons, approximately 168.000 inhabitants, each year were required to report the weekly number of ILI cases aggregated by age group to a local public health centres which were then aggregated at Vojvodina level. From 2010 to 2015, sentinel surveillance covered between 5.2% and 15.8% of total Vojvodina population and included between 89 to 135 sentinel physicians who observed influenza seasons. In the surveillance of ILI, only outpatiens in Health Centres of Vojvodina were covered. From 2010 to 2013 sentinel sites have included počulation of 19 municipalities in Vojvodina, while since 2013 sentinel surveillance was expanded in all of 45 municipalities of Province. The World Health Organization (WHO) and national recommendations, we observed five age groups (0–4, 5–14, 15–29, 30–64 and ≥ 65). General practitioners and paediatricians were included in the network of sentinel physicians and they reported the number of new cases of ILI in their weekly population reference. Also, sentinel physicians electronically entered the data of new cases of ILI weekly and regularly sent samples for virological confirmation to the WHO National Influenza Centre, the Centre for Virology of the Institute of Public Health of Vojvodina in Novi Sad [7].

Hospitalisations SARI and ARDS surveillance

As previously described in detail [7], the hospital coordinators of SARI and ARDS surveillance from all 15 acute care hospitals in Vojvodina sent daily reports on each hospitalized SARI and/or ARDS case to the district coordinators of influenza infection in local departments of public health. Individual reports on the hospitalized cases with SARI and/or ARDS were registered in a computer database in the local departments of public health and in the Institute of Public Health of Vojvodina.

We included patients of all ages who were hospitalized in the intensive care units and high dependency units (severe form of infections), general/internal medicine, pediatric medicine, infectious disease wards and respiratory disease wards in Vojvodina.

Inclusion criteria

The inclusion criteria for the study were clinical diagnosis of ILI, SARI and ARDS. In accordance with the WHO criteria, a case definition as a basis for physicians to collect specimens were following: ILI cases were defined as those with a sudden onset of fever (> 38°C) and cough/sore throat within seven days of the onset, while the inclusion criteria for the SARI patients was defined as presence of an acute respiratory illness with the onset during the previous seven days, and who required overnight hospital admission on the basis of history of fever or measured fever of 38°C, cough, and shortness of breath or difficulty in breathing [9, 10].

ARDS cases were defined as acute onset of bilateral infiltrates on the chest radiograph; arterial oxygen tension partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio < 27 kPa and absence of cardiac failure or left atrial hypertension (assessed clinically, echocardiographically or with invasive monitoring) and required invasive ventilation [11–13].

Laboratory diagnosis

The reference laboratory for virological surveillance of influenza was WHO National Influenza Center, the Centre for Virology of the Institute of Public Health of Vojvodina in Novi Sad [14].

The nasal and throat swabs samples were collected from all patients in Vojvodina who meet the ILI, SARI or ARDS case definition. Once samples were collected, swabs were put in the Viral Transport Media, stored at 4 °C, and then transported to the Centre for Virology and kept at -20 °C. The transport of the samples to the laboratory was organized on a daily basis by local departments of public health. The case data, including demographic and clinical information, were collected on a questionnaire/laboratory form from all patients from whom a swab was collected.

Swab samples from the patients were tested for influenza A (H1N1)pdm09, influenza A (H3N2) and influenza B (without further determination of B/Yamagata-like and B/Victoria-like) virus infection using real-time polymerase chain reaction (RT-PCR) as described previously [15].

The results were analysed using the Applied Biosystems 7500 Software version 2.0.6, and the interpretation of the data was done according to WHO guidelines [16]. Immediately after the testing was finished, the results of the laboratory tested samples were sent to the Institute of Public Health of Serbia, local departments of public health, the sentinel/hospital physicians, and to the patients [7].

Statistical analysis

Using the above-mentioned methodology [7], we calculated weekly incidence rate of ILI in Vojvodina and weekly age-specific incidences of ILI for the monitored age groups were measured per 100,000 of population.

The epidemic threshold of incidence of 246.3/100,000 was determined in the previous 5 pre-pandemic sentinel seasons on the basis of weekly incidence rate of ILI value [7].

To study the evolution of the influenza activity and hospitalization rate during the influenza surveillance seasons, the ILI incidence rates per week and weekly hospitalization rates (number of hospital admissions) were compared for five seasons. Univariate analysis was performed to determine a degree of significance of proposed case definitions related to the laboratory confirmation of the influenza virus. Stepwise logistic regression analysis was performed to determine which case definitions had predicted influenza infection. Odd ratios (OR) and their 95% confidence intervals (CI) were calculated for each variable in the logistic regression model. Comparisons of the influenza A and B virus subtypes distribution by sex, age, proposed case definitions, monthly notified cases and underlying conditions were analysed by Fisher's exact test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was done by using SPSS version 21 software.

Results

Figure 1 (a-d) shows the distribution of influenza type/subtype among all laboratory confirmed cases of influenza. For the five infleunza seasons, the highest value of weekly ILI incidence rate was accompanied by the highest number of confirmed influenza cases. Except the 2013/14 influenza season, when influenza B was not detected, each year, influenza A and B viruses cocirculated. A total of 720 samples were identified as influenza A or B positive. Among the positive cases, 288 (40.0%) were influenza A (H1N1)pdm09, 253 (35.1%) were influenza A (H3N2), 158 (21.9%) were influenza B, while 3.0% of all influenza cases were influenza A without subtypes (these samples had low viral loads that were below the threshold of detection with subtype-specific reagents). The majority of influenza A (H1N1)pdm09 positive samples were detected in the 2010/11 influenza season. In this season, the highest percentage (79.2%; 80/101) of influenza A (H1N1)pdm09 was registered between weeks 4 and 7. The highest proportion of influenza A (H3N2) specimens were registered in the last influenza season (2014/15) when laboratory confirmed 90 cases of influenza A (H3N2) and 20 cases (22.2%) was registered only in the 9/2015 week. During the study period, a total of 158 cases of influenza B were registered and most of influenza B samples were confirmed during 2012/13 influenza season (63.9%; 101/158). In the same influenza season, we registered the highest weekly incidence rate of ILI (712.3/100,000 inhabitants) in the 9/2013 week.

The physician sentinel network data regarding hospitalized cases of influenza and data from virological surveillance for 2010–2015 are presented in the Figure 2. During the study period (2010–2015), the highest weekly incidence rates of ILI were registered during the 2010/11 and 2012/13 influenza seasons. The lowest values of weekly incidence rate were registered in the 2013/14 influenza season. Observed by clinical diagnosis, the majority of cases of SARI and ILI patients with influenza, were registered during the 2012/13 and 2014/15 influenza seasons, respectively. Only during the 2011/12 season we did not detect influenza patients with clinical diagnosis of ARDS.

In the observed period, 1,466 specimens from patients with ILI, SARI or ARDS, were tested for influenza by RT-PCR, and 720 samples were identified as influenza A or B positive (49.1%). Among all the case definitions, the case definition of ILI was a good predictor for influenza confirmation ($p < 0.05$) during five seasons. According to observations regarding each seasons, the patients with SARI or ARDS were more likely to be negative than positive for influenza after testing or were not significantly different ($p > 0.05$) in predicting influenza laboratory confirmation (Table 1).

The duration of the epidemic period was 8 weeks in 2010/11, 3 weeks in 2011/12 and 6 consecutive weeks during 2012/13 and 2014/15. There was no epidemic period in the 2013/14 influenza season, because weekly incidence rate of ILI were below the epidemic threshold.

A total of 38 influenza-associated deaths were reported during the study period. The highest number of deaths (19/38) and number of hospitalized patients (128/402) with influenza were registered during the 2014/15 influenza season, when influenza A (H3N2) (37.8%) and A (H1N1)pdm09 (35.2%) were almost equally present among the confirmed influenza cases. The highest hospitalization rate by week (6.6/100,000) were equally registered during the 2012/13 influenza season (influenza B predominant) as well as during the last one 2014/15. No influenza-associated deaths were reported to us and only 4 patients with influenza were hospitalized during the 2011/12 influenza season when influenza A (H3N2) was predominant (Table 2).

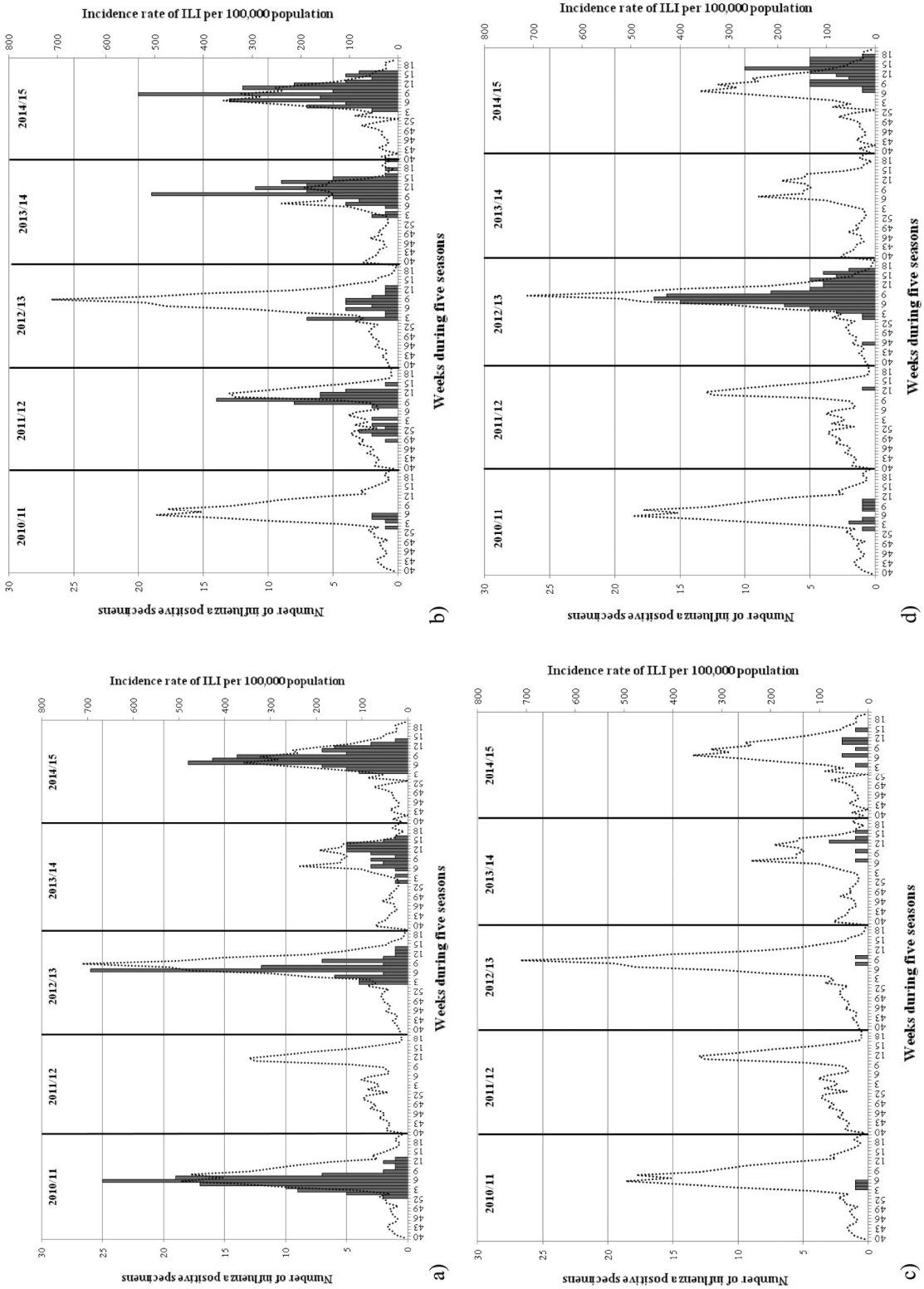

Fig. 1 – The weekly influenze like illness (ILI) incidence rate, the number of influenza positive patients by type/subtype and by week of illness onset in Vojvodina (Serbia) during five consecutive influenza seasons, 2010–2015: a) influenza A (H1N1)pdm09, b) influenza A (H3N2), c) influenza A (non-subtype) and d) influenza B.

Legend: The black vertical lines indicate the separation between different influenza seasons (2010–15).

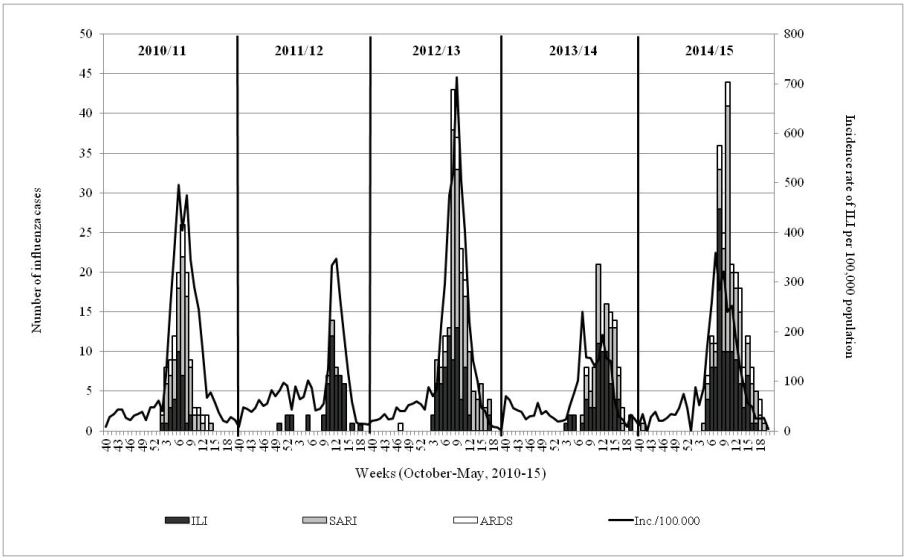

Fig. 2 – The weekly incidence rate and distribution of laboratory-confirmed influenza patients by clinical diagnosis of influenza in Vojvodina (Serbia) during five consecutive influenza seasons, 2010-2015.
Legend: ILI - influenza-like illness; SARI - severe acute respiratory infection; ARDS - acute respiratory distress syndrome.

Table 1

Analysis of the predictors of influenza infection by case definitions in Vojvodina (Serbia) during the investigated period (n = 1,466)

Years	Clinical form, n (%)			
	Total cases	ILI	SARI	ARDS
2010/11				
positive (+)	118 (100.0)	29 (24.6)	66 (55.9)	23 (19.5)
negative (-)	96 (100.0)	9 (9.4)	53 (55.2)	34 (35.4)
OR (95% CI)		3.15 (1.41–7.04)	1.03 (0.60–1.77)	0.44 (0.24–0.82)
p-value		0.0052	ns	0.0096
2011/12				
positive (+)	53 (100.0)	49 (92.5)	4 (7.5)	0 (-)
negative (-)	31 (100.0)	16 (51.6)	7 (22.6)	8 (25.8)
OR (95% CI)		11.48 (3.33–39.6)	0.28 (0.07–1.05)	0.02 (0.01–0.43)
p-value		0.0001	ns	0.0111
2012/13				
positive (+)	199 (100.0)	72 (36.2)	110 (55.3)	17 (8.5)
negative (-)	186 (100.0)	35 (18.8)	131 (70.4)	20 (10.8)
OR (95% CI)		2.45 (1.53–3.90)	0.52 (0.34–0.79)	0.78 (0.39–1.53)
p-value		0.0002	0.0023	ns
2013/14				
positive (+)	117 (100.0)	63 (53.8)	47 (40.2)	7 (6.0)
negative (-)	222 (100.0)	73 (32.9)	125 (56.3)	24 (10.8)
OR (95% CI)		2.38 (1.51–3.77)	0.52 (0.33–0.82)	0.53 (0.22–1.26)
p-value		0.0002	0.0050	ns
2014/15				
positive (+)	233 (100.00)	105 (45.1)	104 (44.6)	24 (10.3)
negative (-)	211 (100.0)	49 (23.2)	117 (55.5)	45 (21.3)
OR (95% CI)		2.71 (1.80–4.09)	0.65 (0.45–0.94)	0.42 (0.25–0.72)
p-value		0.0001	0.0231	0.0017

OR – odds ratio; CI – confidence interval; ILI – influenza-like illness; SARI – severe acute respiratory infection; ARDS - acute respiratory distress syndrome; ns – not significant ($p > 0.05$).

Table 2

Epidemic periods, predominant influenza types, hospitalization rate and number of fatal outcomes by years in Vojvodina (Serbia), 2010–2015 seasons

Parametars	2010/11	2011/12	2012/13	2013/14	2014/15
Duration of epidemic period*	4–11 (8 weeks)	11–13 (3 weeks)	6–11 (6 weeks)	–	6–11 (6 weeks)
Predominant type/subtype of influenza virus	A(H1N1)pdm	A(H3N2)	B	A(H3N2)	A(H3N2)
Number of hospitalized patients with influenza (SARI or ARDS)	89	4	127	54	128
Hospitalization rate **	4.6	0.2	6.6	2.8	6.6
Number of deaths (CFR%)***	6 (6.7)	0 (-)	10 (7.9)	3 (5.6)	19 (14.8)

*** Epidemic period-week incidence rate of influenza like illness (ILI) above 246.3 per 100,000 population; ** Per 100,000 population in Vojvodina according census; ***Case fatality rate (CFR) of hospitalized cases with influenza – severe acute respiratory infection (SARI) or acute respiratory distreses syndrome (ARDS).**

There were a total of 562 influenza A and 158 influenza B virus infections confirmed during the five influenza seasons, without statisticall significant difference between gender regarding the types of influenza virus ($p = 0.1257$).

In the accordance with the proposed case definitions (ILI, SARI or ARS), there were no significant association with a risk of influenza A or B infection among the confirmed cases of influenza ($p > 0.05$).

The predominant age-range of the patients with influenza A (42.2%) and B (43.0%) infection was 30 to 64. The age distribution by the two types of influenza virus showed a significant difference only among the patients of the 15–29 age group ($p = 0.0168$).

Regarding the monthly distribution, influenza A and influenza B predominated during the February-March period, with 76.5% and 82.9%, respectively, in all confirmed cases. During December and January, influenza A type (17.8%) was registered more frequently than influenza B type (7.6%), and the difference was statistically significant ($p = 0.0012$).

The patients hospitalized during five influenza seasons were more likely to present with co-morbidity conditions, such as immunodeficiency, for different reasons.

Table 3

Characteristics of the laboratory-confirmed influenza A and B cases in Vojvodina (Serbia), during the 2010–2015 influenza seasons

Characteristics	Influenza A (n = 562)		Influenza B (n = 158)		*p* values*
	n	%	n	%	
Gender					
male	280	49.8	90	57.0	0.1257
Case definition					
ILI	259	46.1	59	37.3	0.0569
SARI	250	44.5	81	51.3	0.1483
ARDS	53	9.4	18	11.4	0.4529
Age					
0–4	76	13.5	14	8.9	0.1346
5–14	89	15.8	35	22.1	0.0734
15–29	83	14.8	12	7.6	0.0168
30–64	237	42.2	68	43.0	0.8558
≥ 65	77	13.7	29	18.4	0.1619
Months					
October–November	1	0.2	1	0.6	nd
December–January	100	17.8	12	7.6	0.0012
February–March	430	76.5	131	82.9	0.1031
April–May	31	5.5	14	8.9	0.1369
Comorbidities**					
chronic obstructive pulmonary disease	43	7.7	14	8.9	0.6182
diabetes mellitus	32	5.7	13	8.2	0.2643
immunodeficiency	73	13.0	34	21.5	0.0110
any cardiovascular disease	75	13.3	26	16.5	0.3637
chronic nephropathy	35	6.2	9	5.7	1.0000
overweight***	24	4.3	3	1.9	0.2351
pregnancy	12	2.1	1	0.6	0.3173
fatal outcome during hospitalization	30	5.3	8	5.1	1.0000

ILI – influenza-like illness; SARI – severe acute respiratory infection; ARDS – acute respiratory distress syndrome; nd – not determined; *Fisher's exact test; **One patient with confirmed influenza could have one or more comorbidities, simultaneously; ***Body mass index – BMI ≥ 30 kg/m^2.

The immunosuppressed patients were more likely to have influenza B than influenza A infection ($p = 0.0110$). When comparing A and B influenza types, no significant differences were found ($p > 0.05$) regarding chronic obstructive pulmonary disease, *diabetes mellitus*, cardiovascular diseases, chronic nephropathy, obesity, pregnancy and a risk of fatal outcome during hospitalization (Table 3).

Discussion

We conducted the first retrospective surveillance study among outpatients and hospitalized patients with laboratory confirmation of influenza in Vojvodina during the post-pandemic period. Data from our study showed that influenza A (H1N1) pdm09, A (H3N2) and influenza B were detected with different distribution over the observed periods. Three virus subtypes are cocirculating in Vojvodina and the predominant influenza subtypes were influenza A (H3N2) during 3 influenza seasons (2011/12, 2013/14 and 2014/15), influenza B in the 2012/13 season, while influenza A (H1N1)pdm09 was predominant during the first post-pandemic season in Vojvodina (2010/11). These findings are in good agreement with those from previously published results of the WHO reports [17–21].

Similar to the 2012/13 season, a large number of 3 subtypes of influenza cocirculated during the 2014/15 season, as reflected in high hospitalization rate of the patients with SARI and ARDS (6.6 per 100,000 inhabitants) in each of the above-mentioned influenza seasons. During both seasons, epidemic periods lasted for 6 consecutive weeks. The case fatality rate (CFR) in hospitalized influenza cases with SARI or ARDS diagnosis was the highest in the last post-pandemic season (14.8%; 19/128). Except the 2011/12 season, when no influenza-associated deaths were reported, the CFR in hospitalized patients ranged from 5.6% to 7.9% in other influenza seasons. The values of CFR during the post-pandemic influenza seasons was higher than the rates registered during the pandemic season in Vojvodina, when the CFR of hospitalized patients was 2.0% [7]. We believe that by implementing more sensitive hospital surveillance of the novel influenza virus (2009/10 season), it was possible to register more hospitalized patients (1,591) with ARDS, pneumonia, and those with acute febrile illness, than it was possible during the study period when the overall number of hospitalized patients with SARI and ARDS was 402. However, we are convinced that a total burden of influenza is often underestimated because many fatal outcomes are caused by some other secondary complications of influenza.

The case definition of ILI differs from country to country. The sensitivities and specificities of different ILI case definitions are very similar, but the positive predictive value (PPV) and positive likelihood ratio (LR+) are different, with the United States Centers for Disease Control and Prevention (US–CDC) ILI (fever defined as body temperature ≥ 37.8 °C plus cough and/or sore throat in the absence of a known cause other than influenza) having the lowest, and the WHO new ILI (fever defined as body temperature ≥ 38 °C plus cough and with onset within the last 10 days) having the highest PPV and LR+ [22–24].

In the surveillance of influenza in Vojvodina we used WHO's old ILI definition (patients with sudden onset of fever (> 38 °C) and cough/sore throat). The data of some other studies has shown that this case definition provided a slightly smaller different values of PPV and LR+ compared to WHO's new ILI. For example, it was reported that the respective values were 39% and 42% for PPV, whereas those for LR+ were 12.0 and 13.3, respectively [22]. Although there is a controversy as to whether the indicators from previous research are reliable for the estimation of efficacy of ILI case definitions [25], we found more frequently the patients with ILI among confirmed cases compared to the negative results of influenza testing during all five seasons of the sentinel surveillance of influenza. Similarly to the findings of other authors [26], the prevalence of laboratory-confirmed influenza infection in our community, during the five surveillance seasons a percentage of the patients with ILI was 63.6% (318/500). This results show that the sentinel surveillance of ILI and virological surveillance of influenza are an integrated and inseparable system for influenza monitoring.

A higher percentage of negative results of RT-PCR in patients with SARI and ARDS can be explained by the fact that the patients with suspected influenza were hospitalized in the later phases of infection, when PCR isolation of influenza is less sensitive. In accordance with that, the percentage of influenza confirmed cases might be increased if the specimens are collected only during the first few days of the illness. Likewise, the SARI and ARDS cases could be also due to other respiratory viruses, not only influenza [16, 25].

Similar to the results of our research, findings of other studies indicated that clinical features of patients infected with seasonal influenza A and B viruses are similar, despite the fact that the overall influenza A was more commonly detected than influenza B [27, 28].

We found that the influenza B virus circulated throughout the year and detection rates were mainly comparable to those of the influenza A viruses. Nevertheless, when comparing the features of the influenza A and B virus infections among the patients in Vojvodina in the 2010–2015 time frame, it comes out that the influenza A occurred more frequently than influenza B at the beginning of the influenza season (December-January) and more frequently among the 15–29 year old patients. The reasons for the increased number of influenza A positive specimens among young people can be explained by the fact that young people are tested more often [7], and that they, especially children, may shed virus more abundantly and for longer periods than adults [16]. In accordance with mentioned, among all confirmed cases of influenza, the participation of the influenza A virus was higher compared to influenza B virus (the ratio 3.6 : 1.0).

Similarly to the data of some other authors [3], we found that the influenza B followed influenza A as ILI and SARI cases when compared to clinical case definitions of influenza. Likewise, when we compared influenza infections among the patients with ARDS, no differences were found between the frequencies of the two types of influenza.

According to the WHO reports, in the 2010–2015 influenza seasons across Europe and USA, the most commonly

recognized factors for severe disease among the hospitalized adult patients were chronic respiratory disease (or asthma), cardiovascular diseases, metabolic disorders, and obesity. The most commonly reported underlying conditions among hospitalized pediatric patients were asthma, neurologic disorders, and immunodeficiency [17–21]. During the 2014/15 season, the new reports appeared on renal disease with significant presence among adult patients hospitalized because of influenza [21]. The data of the European system for monitoring excess mortality indicated that in the last winter season (2014/15), excess all-cause mortality among the elderly was higher than during the preceding 4 winter seasons [19–21]. In Vojvodina, during the last influenza season, when a large number of all three influenza subtypes were detected, the mortality rate of influenza was 1.0 per 100,000 inhabitants and it was the highest in comparison to the preceding seasons. The higher value of mortality rate of influenza (1.6 per 100,000 inhabitants) in Vojvodina was registered only during the pandemic 2009/10 season [7].

Despite the fact that vaccination plays a key role in controlling influenza transmission among high risk groups as well as in the general population, coverage of vaccination against influenza in Vojvodina remains low and includes mostly population aged 65 years or older [6, 29]. In our study, among cases with influenza-associated deaths, none of the participants had ever received an influenza vaccination.

Our results of the presenting comorbidities were similar in both groups, and the most common were immunodeficiency and cardiovascular disease. In the patients group with the influenza B confirmed infection, immunodeficiency was more commonly detected than in the other groups. A probable explanation for this is that two nosocomial outbreaks (2012/13 and 2014/15) with influenza B were registered, among patients with blood cancer receiving chemotherapy (resulting in immunodeficiency) [29].

Conclusion

The impact of influenza in Vojvodina over the past five influenza seasons has been considerable in terms of surveillance among outpatients as well as among the patients hospitalized with influenza. Earlier respiratory sampling collections for virological evaluation before hospitalization or the modification of actual case definitions of SARI and ARDS could increase the percentage of confirmed cases of influenza among the patients with suspected influenza infection. Besides, increasing number of samples among patients with influenza suspicion infection through the precise laboratory guidelines for sampling would lead to a more precise view on the distribution of influenza A and B viruses in Vojvodina. The activity to increase the coverage of immunization against influenza, particularly among the patients with risk factors for complications of seasonal influenza, could decrease the number of hospitalizations, complications and fatal outcomes due to influenza infection.

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Conflict of interest

The authors declare no conflict of interest.

Ethical standards

All information about patients was anonymised and de-identified. This article does not contain any additional studies with human or animal subjects.

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Morphological characteristics of lateral branches of human basilar artery

Morfološke karakteristike bočnih grana *arteriae basilaris* čoveka

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Abstract

Background/Aim. Lateral branches of basilar artery mostly supply pons and cerebellum. The aim of this study was to determine the morphological characteristics of lateral branches and side branches of the individual lateral branches of human basilar artery. **Methods.** The research was done on 25 anatomical specimens of adult brainstems, both sexes, previously fixed by immersion in a 10% formalin. Microdissection and precise measurements of caliber and length of the basilar artery were done under the stereolupe MBS-9 by the ocular micrometer. **Results.** A number of lateral branches was 9 on both sides, and the number of side branches of the individual lateral branch of basilar artery ranged from 0 to 4. The average diameter of side branches on both sides was 0.15 mm while the average length on the left side was 4.31 mm and 4.06 mm on the right. Side braches on the left side of the basilar artery most commonly penetrated pons and postpontine fossa (29.82% each) and rarely the area of middle cerebellar pedicle (1.74 %). On the right side, side branches most commonly penetrated pons (35.29%), and rarely the areas of inferior and middle cerebellar pedicle (1.96% each). **Conclusion.** Maintrunk of basilar artery gives an equal number of lateral branches on both sides. On average, every lateral branch gives 2 side branches. The most common site of descent of the side branches is pons, while the rarest one was cerebellar pedicle.

Key words:
basilar artery; anatomy; brain stem; pons.

Apstrakt

Uvod/Cilj. Bočne grane *arteriae basilaris* u najvišem procentu vaskularizuju *pons* i *cerebellum*. Cilj rada bio je određivanje morfoloških karakteristika bočnih grana i bočnih ogranaka pojedinačnih bočnih grana *arteriae basilaris* čoveka. **Metode.** Ispitavanje je obavljeno na 25 anatomskih preparata moždanih stabala odraslih osoba, oba pola, prethodno imerziono fiksiranih u 10% formalinu. Mikrodisekcija i precizno merenje kalibra i dužine *arteriae basilaris* i njenih bočnih grana i ogranaka rađeno je pod stereolupom MBS-9, uz pomoć okularnog mikrometra. **Rezultati.** Broj bočnih grana iznosio je 9 na obe strane, a broj bočnih ogranaka pojedinih bočnih grana *arteriae basilaris* kretao se od 0 do 4. Prosečan prečnik bočnih ogranaka na levoj i desnoj strani iznosio je 0,15 mm. Prosečna dužina bočnih ogranaka na levoj strani bila je 4,31 mm, a na desnoj 4,06 mm. Bočni ogranci na levoj strani bazilarne arterije najčešće su ponirali u predelu *pons* i *fossa postpontina* (po 29,82% slučajeva), a najređe u područje *pedunculus cerebellaris medius* (1,74% slučajeva). Na desnoj strani, bočni ogranci najčešće su ponirali u *pons* (35,29% slučajeva), a najređe u područje *pedunculus cerebellaris inferior et medius* (po 1,96% slučajeva). **Zaključak.** Od glavnog stabla *arteriae basilaris* obostrano se odvaja jednak broj bočnih grana. Bočne grane *arteriae basilaris* u najvećem broju slučajeva davale su po dva bočna ogranka. Najčešće mesto poniranja bočnih ogranaka pojedinačnih bočnih grana *arteriae basilaris* obostrano je *pons*, a najređe obostrano, kraci malog mozga.

Ključne reči:
a. basilaris; anatomija; moždano stablo; pons.

Introduction

Basilar artery (BA) arises from the confluence of two vertebral arteries at pontomedullary sulcus between the left and right abducens nerve. It extends to the upper part of the pontine cistern, lying in a shallow groove that limits pyramidal eminence of the pons. Diameter of the BA is mostly constant and below the origin of superior cerebellar artery

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(SCA) is around 4.1 mm. Length of the BA ranges from 15 to 40 mm (32 mm in average). In the caudal part of the interpeduncular cistern, the BA divides into 2 posterior cerebral arteries [1–3]. Number of lateral branches of the BA is somewhat larger on the left side- ranging from 5 to 10 (an average of 8) while on the right side ranges from 4 to 9 (7 in average) [4]. Lateral branches of the BA are: anterior inferior cerebellar artery (AICA), SCA, labyrinthine artery (LA) and many branches for medulla oblongata and pons (pontine arteries). Variations in lateral branches were also seen. For example, posterior inferior cerebellar artery (PICA) may rise from the BA, while the LA may have origin from the SCA [1, 2, 5]. Some authors categorize branches of the BA into paramedian and short and long circumferential arteries. Paramedian arteries originate from the dorsal side of the BA and supply the medial area of the pons, including pontine nuclei, pyramidal and corticonuclear tract, and part of the medial lemnisci. Tegmental, basal and tegmentobasal pontine infarctions are caused by diseases of paramedian branches of the BA. About 60% of infarcts of the pons are paramedian infarctions [1, 6]. Short circumferential branches supply the anterolateral area of the pons. Some branches of these arteries can supply the upper cerebellar pedicle. Long circumferential branches supply the lateral part of the pons, and form anastomoses with branches of the AICAs and SCAs [7, 8].

Detailed knowledge of the morphological characteristics of the BA and its lateral branches significantly contributes to the understanding of the origin and consequences of pathological conditions in areas supplied by these blood vessels.

The aim of the study was to determine the following morphological characteristics of lateral branches of human BA: length, diameter and descending point of lateral branches of the BA; the number of side branches of the individual lateral branches of the BA, distance of their origin from the main trunk of the BA, length, diameter and place of descent of the side branches of the individual lateral branches of the BA.

Methods

The study was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska. The study was carried out on 25 adult brains, both sexes, aged from 35 to 85 years, who died without diagnosed neurological disease. The material was collected at the Department of Pathology, University Clinical Center of the Republic of Srpska. Using conventional autopsy technique, brains were extracted from the cranial cavity, 24–48 hours after death. Out of the brain tissue were allocated brain stem and submerged in 10% formalin solution for 30-day fixation. Fine preparation of arteries was performed with microsurgical forceps and microscissores under the stereoloupe (MBS-9, Carl Zeiss, Germany) by the ocular micrometer with ×8 magnification. Numbering of lateral branches of the BA was carried out from the beginning of the BA (confluence of vertebral arteries) to the bifurcation of the main tree of the BA. The following parameters were measured: diameter at the starting point of lateral branches, their size and location of descent. Accurate measurement of the distance separating the side branches of the lateral branches in relation to the main trunk of the BA and their caliber and length was done under stereolupe and ocular micrometer with the same magnification. One researcher made 3 independent measurements of all analyzed parameters and these results were used to calculate average values. The results were analyzed by the methods of descriptive statistics. Statisical analysis was done with SPSS software version 16 (SPSS Inc, Chicago, USA).

Results

In this study, we found 9 lateral branches of the BA on each side. Figure 1 shows the BA and some of its lateral branches.

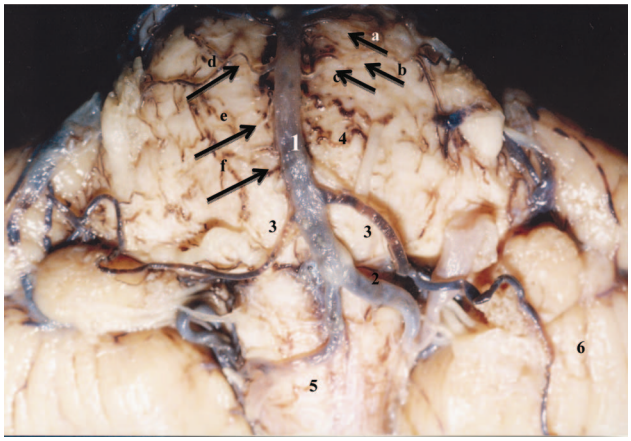

Fig. 1 – Lateral branches of basilar artery (BA): 1– BA, 2-vertebral artery, 3-anterior inferior cerebellar artery (AICA), 4-pns, 5-medulla oblongata, 6-cerebellum. The arrows: a-eighth left lateral branch; b –seventh left lateral branch; c - sixth left lateral branch; d- fifth right lateral branch; e - fourth right lateral branch; f - third right lateral branch.

The average length of lateral branches of the BA in the anatomic samples fixed in formalin was 9.98 mm on the left side and 10.11 mm on the right side. The average diameter of the starting points of lateral branches of the BA on the left side WAs 0.41 mm, while on the right side was 0.42 mm. Lateral branches of the BA in most cases both sides penetrated into the area between the midbrain and cerebellum.

The average values of morphological parameters of all 9 lateral branches of the BA, level of origin from the beginning of the BA, length, diameter and place of descent are presented in Table 1. Number of side branches of the individual lateral branches of the BA ranged from 0 to 4, an average of 2 side branch. The average diameter of the side branches on the left and the right side was 0.15 mm. The average length of the side branches on the left side was 4.31 mm, while on the right was 4.06 mm. Side branches on the left of the BA in 29.82% of cases penetrated pons and postpontine fossa, in 14.03% prepontine fossa, in 8.77% cerebellum and the basilar sulcus, in 7.01% root of the trigeminal nerve and in 1.74% of cases area of the middle cerebellar peduncle. Side branches on the right side of the BA most commonly penetrated pons, in 35.29% of cases, followed

by postpontine fossa in 25.49% of cases, prepontine fossa in 15.68% of cases, basilar sulcus in 9.8% of cases, cerebellum in 5.88% of cases, the root of the trigeminal nerve in 3.92% of cases and areas of middle and inferior cerebellar peduncle in 1.96% of cases each.

The first left lateral branch originated at the average distance of 2.91 mm (range 0.5–6.7) from the beginning of the BA. Its length was 7.4 mm on average (range 2.1–12.5), and diameter 0.3 (average 0.1–1.2 mm). The first right side branch originated somewhat nearer the beginning of the BA – 2.77 mm (range 0.5–7.5); it was longer than the first left side branch (8.38 mm, range 2.1–26.5) while the diameter was pretty similar 0.29 mm (range 0.1–1.1). Bilaterally the first branches of the BA penetrated the postpontine fossa.

Most commonly, lateral branches of the BA showed no side branches. Eight lateral branch on the right side did not give any side branches in all analyzed samples. Eight left and ninth right lateral branches in 92% of each did not give side branches. Second left lateral branch of the BA most commonly gave side branches (Table 2).

Table 1

Morphological characteristics of lateral branches of the basilar artery (BA)

Lateral branches	Level of separation from the beginning of the BA (mm)	Length (mm)	Diameter of lateral branches origin point (mm)	Place of descent
I				
left	2.91 (0.5–6.7)	7.41 (2.5–12.5)	0.3 (0.1–1.2)	Postpontine fossa
right	2.77 (0.5–7.5)	8.38 (2.1–26.5)	0.29 (0.1–1.1)	
II				
left	5.86 (2.3–11.8)	12.75 (1–36.5)	0.44 (0.1–1)	Cerebellum
right	5.42 (1.1–11.3)	15.95 (1.2–44.4)	0.55 (0.2–1.1)	
III				
left	8.35 (5.7–13.3)	10.02 (0.2–33.4)	0.43 (0.1–1.5)	Pyramidal eminence
right	7.97 (2.6–15.4)	8.74 (0.7–32.4)	0.34 (0.1–0.9)	
IV				
left	10.34 (6.2–14.5)	9 (1–32.3)	0.25 (0.1–0.7)	
right	10.45 (5.6–16.1)	7.63 (0.5–27.4)	0.23 (0.1–0.5)	
V				
left	12.51 (8.6–16.7)	6.94 (0.2–13.5)	0.27 (0.1–0.8)	
right	12.29 (7.1–17.8)	6.53 (0.5–13.6)	0.24 (0.1–0.5)	
VI				
left	13.37 (9.7–17.1)	8.79 (1.2–19.4)	0.33 (0.1–0.9)	Between midbrain and cerebellum
right	13.47 (8.6–18.1)	10.45 (1.1–14.7)	0.28 (0.1–0.9)	Lateral side of the pons
VII				
left	13.71 (10.4–18.1)	10.79 (2.5–17.6)	0.43 (0.1–1)	Between midbrain and cerebellum
right	14.34 (10.1–18.3)	9.55 (7.1–18.5)	0.55 (0.2–1)	
VIII				
left	14.86 (11.2–18.4)	11.79 (2.1–16.2)	0.53 (0.1–1)	
right	15.22 (11.2–18.3)	10.45 (5.1–17.1)	0.61 (0.2–0.9)	
IX				
left	15.66 (13.4–18.6)	12.34 (7.4–16.2)	0.72 (0.3–0.9)	
right	14.8 (12.2–18.5)	13.37 (13.1–13.6)	0.73 (0.6–0.9)	

***Note: the values are given as mean values (minimum–maximum).**

Table 2

Percentage of cases in each lateral branch without side branches

Side	Unbranched lateral branch (%)								
	I	II	III	IV	V	VI	VII	VIII	IX
left	76	48	56	88	88	80	80	92	88
right	76	68	64	64	84	80	72	100	92

Note: I–IX – number of lateral branches.

Morphological characteristics of lateral branches of the BA which gave only one side branch are presented in Table 3. The fourth right lateral branch most frequently (32%) gave one side branch, while the ninth right and left lateral branches rarely gave one side branch (4% each). The shortest distance of origin was seen in first left side branch (4.1 mm), while the longest distance was seen in the second right side branch (21.38 mm). Regarding the length, the shortest side branch was the sixth on the right side (1.63 mm), while the longest was the third right side branch (9.48 mm). The diameter of side branches ranged from 0.1 to 0.3 mm.

Regarding the presence of 2 side branches, the fifth and the eight lateral basilar branch showed no side branches on both sides. Other arteries more commonly had side branches on the left side. The second left and the third right lateral branches of the BA, most commonly, gave two side branches (Table 4).

Table 3

Morphometric characteristics of cases where lateral branch of the basilar artery (BA) gave only one side branch (arterial twigs)

Parameter	Arterial twigs, left/right (%)								
	I (16/16)	II (16/16)	III (28/20)	IV (12/32)	V (12/12)	VI (12/16)	VII (16/16)	VIII (8/0)	IX (4/4)
ADI (mm)	4.1 (3.7–7.5)/ 4.15 (1.1–6.4)	13.15 (3.4–21.3)/ 21.38 (0.5–42.4)	8.76 (7.4–12.3)/ 5.92 (0.3–9.1)	5.06 (1.2-10.6)/ 6.06 (0.4-12.1)	9.66 (7.6–11.5)/ 4.13 (1.4–9.5)	8.5 (1–12.5)/ 6.75 (3.5–11.2)	7.42 (3.2–14.2)/ 7.35 (5.5–9.4)	5.65 (4.5–6.8)/ 0	7.2 (2–7.2) / 5.6 (4.5–6.8)
AL (mm)	3.73 (1.2–5.7)/ 2.68 (1.5–4.5)	4.25 (0.5–6.8)/ 4.98 (2.1–9.7)	4.56 (1.2–9.5)/ 9.48 (1.5–31.5)	2.13 (0.7–4.5)/ 2.53 (1.2-6.4)	2.33 (2.2-2.4)/ 3.13 (1.3-6.8)	3.46 (0.6–7.5)/ 1.63 (0.6–3.2)	6.32 (2.5–13.2)/ 6.03 (1.7–8.5)	4.2 (2.5–5.9)/ 0	5.7 (2.4–5.7)/ 7.5 (7.1–7.9)
AD (mm)	0.13 (0.1–0.2)/ 0.2 (0.1–0.3)	0.2 (0.1–0.4)/ 0.18 (0.1–0.3)	0.13 (0.1–0.2)/ 0.2 (0.1–0.5)	0.1 (0.1)/ 0.13 (0.1–0.2)	0.13 (0.1–0.2)/ 0.1 (0.1)	0.13 (0.1–0.2)/ 0.15 (0.1–0.2)	0.17 (0.1–0.2)/ 0.18 (0.1–0.3)	0.2 (0.1-0.3)/ 0	0.2 (0.1-0.2)/ 0.3 (0.2-0.3)
EP (%)	8 postPF; 8 BAS/ 16 postF	8 postPF; 4 P; 4 C/8 postPF; 4 BAS; 4 C	8 postPF; 16 P; 4 BAS/20 P	8 P; 4 BAS/ 24 P; 4 BAS; 4 rV/	8 P; 4 rV/8 P; 4 prePF	8 P; 4 prePF/4 postPF; 12 P	8 P; 4 prePF; 4 BAS/4 prePF; 8 P; 4 C	4 P; 4 prePF/0	4 prePF/4 P

ADI – average distance of origin of side branch: a distance from the origin of lateral branch from the main trunk of the BA to the beginning of side branch; AL – average length; AD – average diameter; EP – emerging point; P – pons; C – *cerebellum*; prePF – *prepontine fossa*; postPF – *postpontine fossa*; BAS – BA sulcus; rV – root of the trigeminal nerv; ICP – *inferior cerebellar peduncle*; MCP – *middle cerebellar peduncle*.
Note: Arterial twigs is term for one side branch.
All values are given as mean (minimum–maximum) or percentage.

Table 4

Morphometric characteristics of cases where lateral branch of the basilar artery (BA) gave two side branches

Parameter	Two side branches, left/right (%)						
	I (8/4)	II (24/8)	III (8/12)	IV (0/4)	VI (8/4)	VII (4/12)	IX (0/4)
Left, 1st/2nd							
ADI (mm)	4.35 (4.2-4.5)/ 5.8 (5.1–6.5)	5.7 (3.4–7.2)/ 9.95 (5.1–21.2)	4.2 (1–7.4)/ 9.4 (9.2–9.6)	0/0	2.75 (1–4.5)/ 6.15 (4.5–7.8)	8.5 (8.5)/ 8.8 (8.8)	0/0
AL (mm)	4.35 (3.5–5.2)/ 4.95 (4.8–5.1)	3.98 (3.5–7.5)/ 3.93 (1.5–5.1)	2.35 (1.2–3.5)/ 5.2 (4.2–6.2)	0/0	2.4 (0.6–4.2)/ 4.3 (4–4.6)	4.1 (4.1)/ 4.8 (4.8)	0/0
AD (mm)	0.15 (0.1–0.2)/ 0.15 (0.1–0.2)	0.2 (0.1–0.4)/ 0.15 (0.1–0.2)	0.15 (0.1-0.2)/ 0.25 (0.2-0.3)	0/0	0.1 (0.1)/ 0.15 (0.1-0.2)	0.2 (0.2)/ 0.2 (0.2)	0/0
EP (%)	8 postPF/8 postPF	20postPF 4 P/12 postPF 8 P; 4	8 P/4 P 4 MCP	0/0	4% P 4 BAS/4rV 4prePF	4 rV/4rV	0/0
Right, 1st/2nd							
ADI (mm)	7 (7)/ 9.5 (9.5)	2.3 (0.5–4.1)/ 3.85 (2.8–4.9)	8.66 (0.8–17)/ 11.83 (7.6–19.5)	10.5 (10.5)/ 12.3 (12.3)	3.5 (3.5)/ 5.1 (5.1)	7.23 (5.5-9.4)/ 9.73 (7.2-12.6)	4.5 (4.5)/ 10.8 (10.8)
AL (mm)	3.2 (3.2)/ 3.5 (3.5)	3.6 (2.1-5.1)/ 4.95 (4.3-5.6)	4.5 (1.7-8.2)/ 6.2 (2.4-9.6)	6.4 (6.4)/ 3.7 (3.7)	2.1 (2.1)/ 1.6 (1.6)	2.26 (1.7-3.4)/ 2.36 (2.1-2.6)	7.9 (7.9)/ 5.1 (5.1)
AD (mm)	0.1 (0.1)/ 0.1 (0.1)	0.2 (0.2)/ 0.25 (0.2-0.3)	0.16 (0.1-0.2)/ 0.25 (0.2-0.3)	0.2 (0.2)/ 0.1 (0.1)	0.1 (0.1)/ 0.1 (0.1)	0.13 (0.1-0.2)/ 0.13 (0.1-0.2)	0.2 (0.2)/ 0.2 (0.2)
EP (%)	4 postPF/ 0.1 (0.1)	4 postPF; 4 BAS/4 postPF 4P	4 P; 4 C 4 BAS/4postPF 4 P; 4 rV	4 MCP/ postPF	4 prePF/4 prePF	8 prePF 4 P/8prePF 4 P	4 P/4 P

For abbreviations see under Table 3.

Regarding the presence of 3 or 4 side branches, in most studied samples they were absent. The second right and the ninth left lateral branch gave three side branches in 8% of each, while the fourth, sixth, seventh and eighth lateral branch did not give three side branches at all. Second left side branch gave four side branches in 8% of cases, third on the left side in 4% of cases, while other lateral branches did not give four side branches (Table 5).

Table 5

Presence (in percentages) of three and four side branches of individual lateral basilar artery (BA) branch

Parameter	Individual lateral BA								
	I	II	III	IV	V	VI	VII	VIII	IX
Three side branches									
left	0	4	4	0	0	0	0	0	8
right	4	8	4	0	4	0	0	0	0
Four side branches									
left	0	8	4	0	0	0	0	0	0
right	0	0	0	0	0	0	0	0	0

Discussion

BA gives important perforating branches into the pons. Cranial part of the BA is filled by internal carotid artery through the posterior communicating arteries [4]. Demel and Broderick [9], divided the BA into 3 segments: the proximal, middle and distal segment. The distal segment is divided into posterior cerebral arteries that supply the occipital lobes, the lower parts of the temporal and medial parts of parietal lobes and thalamus. Other important branches of the BA are the SCA and AICA. Dodevski et al. [10], reported that the SCA separated from the distal segment of the BA in 96.33% of the cases. The authors presented a possibility of unilateral duplication of the SCA in 1.83% of cases which was not confirmed in the results of our study.

In the current literature there is a little amount of data about the morphology of lateral branches of the BA. Available information relates to the "rough" morphology of blood vessels, without their detailed description [11]. In our previous study, we examined the morphological characteristics of the main tree of the BA. We found that the average length of the BA was 27.63 mm and the diameter 3.16 mm [4]. Saeki and Rhoton [12] published similar results: in their samples, the average length of the BA was 32 mm, diameter 4.1 mm and number of lateral branches was 8 on average. In our samples we described 9 lateral branches of the BA on the left and right side. According to the place of descent and the level of separation from the BA, 9 lateral branch could be marked as the SCA. Marinković et al. [13] analyzed the perforating branches of the BA and divided them into 3 groups: caudal (lateral branches which originate from the beginning of the BA to the place of origin of the AICA), middle (lateral branches which originate from the beginning of the AICA to the rostral third of the BA) and rostral (branches from the terminal part of the BA). They described 2 to 5 caudal perforating branches (out of which 1 or 2 originating from the AICA), 5 to 9 perforating branches originating from the middle group, while 1 to 5 originating from rostral part of the BA (out of which 1 or 2 originating from the first part of the SCA). Range of diameters was 0.08 to 0.9 mm [13]. The average diameter of these branches in our study was 0.725 mm, which is somewhat similar to the results of Saeki and Rhoton [12] who published the range of diameter of lateral branches from 0.1 to 0.5 mm [12]. Dodevski et al. [10] had significantly larger average diameter in their study of 1.42 mm. The differences could be explained by the different number of samples and visualization technique.

Diseases of blood vessels are the most common causes of death in human population. Changes in brain vessels are not only caused by age, but also by an increasing number of risk factors and a modern way of life [11, 14]. Patients with the untreated BA stenosis have a bad prognosis, with the posibility for infarction in 50% of patients within the first 2 years [15]. Ćulafić et al. [16] showed a case of a patient with 85% of the BA stenosis, located in the caudal segment of the BA, 3.27 mm in length. Knowledge of the morphological characteristics of the BA, its lateral branches and side branches is important in understanding the ischemic lesions of the cerebellum, pons and medulla oblongata. The incidence of ischemic diseases of the brain, caused by the occlusion of the arteries of the posterior segment of Willis hexagon, is 15–20% of cases and ischemia caused by the BA occlusion is reported in 1–4% of cases in respect of all ischemic brain lesions [9, 17]. Distally from the place of occlusion, the BA can be filled through the vertebral arteries [18]. Paramedian pontine infarction, caused by thrombosis and arterial perforation, manifests as facial hemiparesis with dysarthria and somatosensory disorders. Motor damage in paramedian basal infarction follows the topographic distribution of the pyramid tracts. Due to somatotophic organization of corticospinal tract at the base of the pons, fibers in the upper part of the pons are not affected by paramedian ischemia, and therefore such patients have better prognosis than those with infarction in the lower part of the pons [19, 20]. Park et al. [21] examined morphological characteristics of talamoperforating arteries and their clinical significance in the field of neurosurgery and neurology. Their research emphasized the necessity of precise knowledge of morphological characteristics of the BA and its lateral branches.

Since formalin fixation might cause some retraction or shrinkage of the tissue, samples used in this study do not replicate completely the tissue response *in vivo*. Although we

made every effort to avoid side effects of such fixation, it was difficult to predict how they would influence the results. It could be useful to compare the results of morphometric studies made *in vivo* and on formalin-fixed samples in order to determine the reproducibility and reliability of results in cadaveric samples. Also, samples in this study were fixed without previous perfusion and impregnation with omnipaque and gelatine, which might have caused differences in length and diameter of vessels compared to the other studies.

Conclusion

Number of lateral branches of the BA was 9 on the left and the right side. The average length of lateral branches of the BA in anatomic samples fixed in formalin on the left side was 9.98 mm, and 10.11 mm on the right side. The average diameter of the starting points of lateral branches of the BA on the left side was 0.41 mm, while on the right was 0.42 mm. Lateral branches of the BA in most cases on both sides penetrate into the area between the midbrain and cerebellum.

Number of side branches of the individual lateral branches of the BA ranged from 0 to 4, an average of 2 side branches.

The average diameter of the side branches on the left and the right side was 0.15 mm. The average length of the side branches on the left side was 4.31 mm, while on the right side was 4.06 mm.

Side branches of the lateral branches on the left side of the BA in most cases, plunged into the pons and fossa postpontina and on the right side of the BA they plunged into the pons.

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Platelet turnover and function in end-stage renal disease

Promet i funkcija trombocita u završnom stadijumu hronične bubrežne insuficijencije

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Abstract

Background/Aim. End-stage renal disease (ESRD) is characterized by significant impairment of platelet functions which may cause bleeding or thrombotic complications. The iam of this study was the aim of this study was the assessement of platelet turnover and function and their correlation with inflammatory and procoagulant markers in ESRD patients as well as platelet indicies comparison between ESRD diabetic and ESRD non-diabetic patients. **Methods.** The prospective, observational clinical study included 63 ESRD patients and 30 age and sex matched healthy volunteers. Following laboratory parameters of platelet turnover and function (platelet count, reticulated platelets, platelet indices, whole blood impedance platelet aggregation), inflammatory and procoagulant markers (number of neutrophils, neutrophil to lymphocyte ratio, C-reactive protein, plasma fibrinogen, D dimer, von Willebrand factor) were obtained. **Results.** Platelet turnover (% of reticulated platelets) was significantly higher (3.8 ± 2.3 vs. 2.3 ± 1.3; $p < 0.01$) and platelet aggregation tests induced by thrombin receptor activiting peptide (TRAP) ($p < 0.01$), adenosine diphospate (ADP) ($p < 0.05$), arachidonic acid (ASPI) ($p < 0.05$) and collagen ($p < 0.05$) were markedly increased in the ESRD patients compared to the control group. The comparison of chronic inflammation and procoagulant markers revealed higher values in all patients comparing to the group of healthy subjects ($p < 0.01$ regarding all parameters). There was no difference between the ESRD diabetic and ESRD non-diabetic patients. **Conclusion.** Results point out increased platelet turnover in ESRD as a consequence of platelet activation and consumption induced by clotting system hyperactivity and chronic inflammation. None of the examined parameters do not predict bleeding occurrence.

Key words:
kidney failure, chronic; diabetes mellitus; platelet funcion tests.

Apstrakt

Uvod/Cilj. Završni stadijum hronične bubrežne insuficijencije [end-stage renal disease (ESRD)] karakteriše prisustvo značajnog oštećenja trombocitnih funkcija koje mogu dovesti do krvarećih i tromboznih komplikacija. Cilj rada bio je procena prometa i funkcionalnosti trombocita i korelacija sa zapaljenskim i prokoagulantnim markerima kod bolesnika sa ESRD, kao i poređenje trombocitnih indeksa između bolesnika kod kojih je šećerna bolest etiološki faktor ESRD i bolesnika sa ESRD druge etiologije. **Metode.** U prospektivno opservaciono kliničko istraživanje uključeno je 63 ispitanika sa ESRD i 30 zdravih osoba usklađenih po starosti i po polu (kontrolna grupa). Određivani su laboratorijski parametri prometa i funkcionalnosti trombocita (broj trombocita, retikulisani trombociti, trombocitni indeksi i agregabilnost trombocita merena impendancom iz pune krvi), zapaljenski i prokoagulantni markeri (broj neutrofila, odnos neutrofila i limfocita, C-reaktivni protein, fibrinogen, D dimer, von Willebrand-ov faktor). **Rezultati.** U grupi ispitanika sa ESRD u poređenju sa grupom zdravih, promet trombocita (% retikulisanih trombocita) je bio statistički značajno veći (3,8 ± 2,3 vs. 2,3 ± 1,3; $p < 0,01$), kao i agregabilnost trombocita indukovana sa trombin receptor-aktivirajućim peptidom (TRAP) ($p < 0,01$), adenozin-difosatom (ADP) ($p < 0,05$), arahidonskom kiselinom (ASPI) ($p < 0,05$) i kolagenom ($p < 0,05$). U ESRD grupi su potvrđene značajno više vrednosti svih zapaljenskih i prokoagulantnih markera u odnosu na zdrave ispitanike ($p < 0,01$, za sve parametre). Nije utvrđena razlika u ispitivanim parametrima između grupe bolesnika kod kojih je šećerna bolest uzročnik ESRD i grupe bolesnika sa ESRD druge etiologije. **Zaključak.** Rezultati pokazuju da je povišen promet trombocita u ESRD posledica njihove aktivacije i potrošnje indukovane hiperaktivnošću koagulacionog sistema i hroničnim zapaljenjem. Nijedan od ispitivanih parametara ne predviđa mogućnost pojave krvarenja.

Ključne reči:
bubreg, hronična insuficijencija; dijabetes melitus; trombociti, funkcijski testovi.

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Introduction

Cardiovascular diseases (CVD) are the most common cause of mortality among patients with chronic kideny disease (CKD). Due to progressive development of atherothrombosis, the risk of CVD in patients with impaired renal function is up to twentyfold higher as compared to healthy subjects [1, 2]. On the other hand, the association of CKD with different clinical forms of hemorrhagic diathesis is well-known [3, 4]. Thrombocytopathia and shortened life span of platelets in the uremic milieu are caused by impaired thrombocytopoiesis, structural and functional disorders and increased platelet consumption ("turnover") due to the activation of haemostatic system and the presence of chronic inflammation in CKD. The above-mentioned changes appear as an increased number of reticulated platelets (platelet with enhanced RNA), among other laboratory findings [5, 6]. Considering the role of primary haemostasis in the process of bleeding and atherothrombosis and the fact that the progressive course of CKD is characterized by the presence of clinically quite opposite but equally represented manifestation of a complex disorder of haemostasis – the paradoxical coexistence of prothrombotic state and bleeding tendency, the evaluation of altered platelet function in conditions of chronic inflammation and activation of the coagulation system could contribute to the elucidation of the pathophysiological mechanisms of atherothrombosis, CVD and bleeding diathesis in chronic kidney disease [7, 8].

With respect to the fact that many of the biological parameters in the diabetes mellitus (DM) and CKD are altered and that both diseases have a high risk of atherothrombosis and CVD, we thought it would be useful to examine whether the changed functionality of primary hemostasis was dominantly influenced by ESRD or by DM as etiological factor [9].

In the absence of a single predictor marker, we hypothesized that the usage of one of the platelet indices (e.g. reticulated platelets, mean platelet volume, plateletcrit and plateled distribution width) in addition to their total number and aggregability, could be used as an auxiliary laboratory method in identification of patients with higher susceptibility to the occurrence of one of the two above-mentioned clinically “opposite” disorders of haemostatic system functionality in CKD-bleeding or thrombosis [10, 11].

Methods

The study was conducted at the Clinical Centre of Vojvodina, Novi Sad in accordance with the Helsinki Declaration, approved by the local Ethics Committee, with the written consent of all the participants who were also interviewed. Observational clinical trial was initiated in November, 2012 and concluded on 31st of March, 2015. It included the total of 93 subjects divided into two groups: Group I included 63 patients with ESRD and with the intention of separate study on DM impact on investigated parameters; it was divided into two subgroups: the first, labelled as IA, consisted of 25 patients having DM as the cause of ESRD while the second one was labelled as IB with 38 patients whose ESRD etiology was based on some other factors (hypertension, glomerulonephritis, polycystic kidney disease, etc.). Group II was the control group included 30 age and sex matched healthy volunteers, non-smokers who did not use any drugs.

The study included both men and women with ESRD (glomerular filtration rate – GFR < 15 mL/min 1.75 m², Cockcroft-Gault equation) hospitalized prior to the creation of permanent vascular access for haemodialysis (autologous arteriovenous fistula) without contraindications for surgery. The study excluded patients younger than 18, pregnant women, those who did not give consent to participate in the study, those who had associated malignancy, individuals with liver failure and those with an acute complication of DM.

Blood samples for laboratory investigations were obtained in the morning after overnight fasting by puncture of the cubital vein and were analyzed within 120 minutes.

For the platelet indices determination, the blood samples were collected in vacuum tubes containing K_2EDTA. For the analysis of platelet aggregation, the blood samples were taken in vacuum blood collection tubes containing Li-heparin, and for the determination of fibrinogen, D dimer, vWF Ag and vWAct blood was extracted in the vacuum tube with Na-citrate.

As a part of complete blood count (CBC), number of neutrophils (NoN), neutrophil to lymphocyte ratio (NLR**)**, platelet count (NoPlt), reticulated platelets (% rPlt) and platelet indices – mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) were measured by automated hematology analyser CELL-DYN Sapphire, Abbott Diagnostics, using fluorescent flow cytometry analyzer to provide automated reticulocyte analysis and separate subpopulations of young cells (reticulated platelets are an integral part of reticulocyte essays) of mature blood cells [12].

The degree of platelet aggregation was evaluated using whole blood impedance platelet aggregometer, Multiplate®; Dynabyte, Munich, Germany, in basal conditions – thrombin receptor activating peptide (TRAP) test, and with adenosin-diphosphate (ADP), arachidonic acid (ASPI) and collagen as agonists (20 μL of each reagent).

The values of plasma fibrinogen (FBG), D dimer, von Willebrand factor activity (vWF Act) and von Willebrand factor antigen (vWF Ag) were determined using ACL analyzer, Instrumentation Laboratory Assays, Italy.

The value of blood glucose, urea, creatinine and C-reactive protein (CRP) was determined by using automatic biochemical analyzer Architect c8000, Abbott Diagnostics.

Body weight and height were measured and body mass index (BMI) was calculated using the formula: BMI = Body weight (kg)/height (m) ^{2}.

Data distribution was tested by the Kolmogorov-Smirnov test. Normally distributed data was presented as the mean ± SD and as the median (25th, 75th percentile) if not normally distributed. Two-sided unpaired *t* test was used for comparison of means between the groups and Mann-Whitney test was used to compare the median values between groups if data was not normally distributed. Correlations between various parameters were determined by Pearson's correlation analysis. Categorical variables were compared by χ^2 test. A *p*-value < 0.05 was con-

sidered to be statistically significant. Statistical software used for the statitical anaysis was MedCalc® Ver. 12.1.3 (MedCalc software, Mariakerke, Belgium).

Results

There was no statistically significant difference between the group of patients with ESRD and healthy control regarding age, gender and BMI. Blood glucose, urea and creatinine concentration were significantly higher in the patients' group.

The comparison of demographic characteristics examined in the defined IA and IB subgroups of patients revealed no differences. Blood glucose concentration was significantly higher in the Group IA DM ESRD while urea and creatinine were not (Table 1).

Table 1

Baseline characteristics and biochemical parameters (blood glucose, urea and creatinene concentration) of study population

Parameter	Group I ESRD (n = 63)	Group II CG (n = 30)	I A DM ESRD (n = 26)	I B non-DM ESRD (n = 37)	*p*-value	*p**-value
Age (years)	60.9 ± 11.7	56 ± 11.1	62.1 ± 10.9	60.0 ± 12.3	ns	ns
Male, n (%)	47 (74.6)	21 (70)	18 (69.2)	29 (78.4)	ns	ns
Female, n (%)	16 (25.4)	9 (30)	8 (30.8)	8 (21.6)	ns	ns
BMI (kg/m²)	26.7 ± 5.4	25.1 ± 3.2	25.6 ± 3.9	27.4 ± 6.2	ns	ns
Blood glucose (mmol/L)	6.4 ± 3.1	5.1 ± 0.5	8.1 ± 4.0	5.2 ± 1.4	< 0.01	< 0.01
Urea (mmol/L)	28.1 ± 10.3	5.0 ± 1.2	29.0 ± 7.6	27.5 ± 11.9	< 0.01	ns
Creatinine (μmol/L)	686.4 ± 312.2	79.7 ± 17.6	618.5 ± 198.4	734.2 ± 367.2	< 0.01	ns

The data are expressed as mean ± SD; sample size – n (%); ESRD – end-stage renal disease; CG – control group; DM-diabetes mellitus; non-DM – non diabetes mellitus; BMI – body mass index; *p*-value, difference between Group I ESRD/Group II CG; *p-value, difference between subgroup IA DM ESRD/subgroup IB non-DM ESRD.**

Also, there was no difference in platelet count (NoPlt) and platelet indices (MPV, PDW, PCT) between the two groups, but the value of reticulated platelets (% rPlt) was significantly higher in the ESRD group than in the healthy volunteers group (3.8 ± 2.3 vs. 2.3 ± 1.3; $p < 0.01$) (Table 2).

Furthermore, the platelet aggregation tests induced by TRAP, ADP, ASPI and collagen were significantly higher in the ESRD patients compared to the control group. The comparison of chronic inflammation and procoagulant markers revealed significantly higher values of fibrinogen (5.0 ± 1.4 vs. 3.3 ± 0.6 g/L; $p < 0.01$), the number of neutrophils (5.4 ± 2.3 vs. 3.2 ± 1.1 × 10^9/L; $p < 0.01$), NLR [3.2 (2.3–4.9) vs. 1.5 (1.2–1.9); $p < 0.01$], CRP [15.1 (3.5–42.4) vs. 0.9 (0.4–1.4) mg/L]; $p < 0.01$], D dimer [738.0 (355.0–1070.5 ng/mL) vs. 192.0 (118.3–246.3); $p < 0.01$], vWF Ag (215.8 ± 78.3 vs. 142.5 ± 41.6%; $p < 0.01$) and vWF Act (170.0 ± 57.9 vs. 120.6 ± 36.7%; $p < 0.01$) in the observed group compared to the group of healthy subjects.

Considering IA and IB subgroups, only the number of platelets in the subgroup of the DM ESRD was higher compared to the IB non-DM ESRD subgroup but this differencedid not reach statistical significance (277.3 ± 108.9 vs. 228.0 ± 108.9; $p = 0.08$). Interestingly, the values of all other examined parameters were not statistically significantly different.

In order to evaluate the association between platelet turnover and function with inflammatory and procoagulant markers in the ESRD patients and their relationship with diabetes mellitus as the most frequent etiological factor of chronic kidney disease correlation analysis was carried out. We found a significant positive correlation between platelet count and number of neutrophils ($r = 0.391$, $p < 0.01$), serum fibrinogen ($r = 0.440$, $p < 0.01$) and CRP concentration ($r = 0.264$; $p < 0.05$) and the absence of correlation of any other platelet indices with inflammatory and procoagulant markers in the ESRD patients. Furthermore, in the same group, there was a significant positive correlation between enhanced platelet aggregability and the number of neutrophils [with TRAP ($r = 0.387$, $p < 0.01$), ASPI ($r = 0.321$, $p < 0.05$), ADP ($r = 0.366$, $p < 0.01$) and collagen ($r = 0.281$, $p < 0.05$) as agonists] and between the increased platelet aggregation and concentration of inflammatory markers i.e. fibrinogen [with ASPI ($r = 0.309$, $p < 0.05$), ADP ($r = 0.260$, $p < 0.05$) and collagen ($r = 0.290$, $p < 0.05$)], and CRP [with ASPI ($r = 0.294$, $p < 0.05$) and ADP ($r = 0.302$, $p < 0.05$) as inductors] (Figure 1).

In the DM ESRD subgroup a significant positive correlation between platelet count and the number of neutrophils ($r = 0.485$, $p < 0.05$) and CRP ($r = 0.444$, $p < 0.05$), and between platelet aggregation and fibrinogen concentration [ADP ($r = 0.400$, $p < 0.05$) and collagen ($r = 0.415$, $p < 0.05$)] was found. In the non-DM ESRD subgroup positive correlations between platelet count and serum fibrinogen ($r = 0.356$, $p < 0.05$) and between platelet aggregability and CRP [ASPI ($r = 0.392$, $p < 0.05$)] were present.

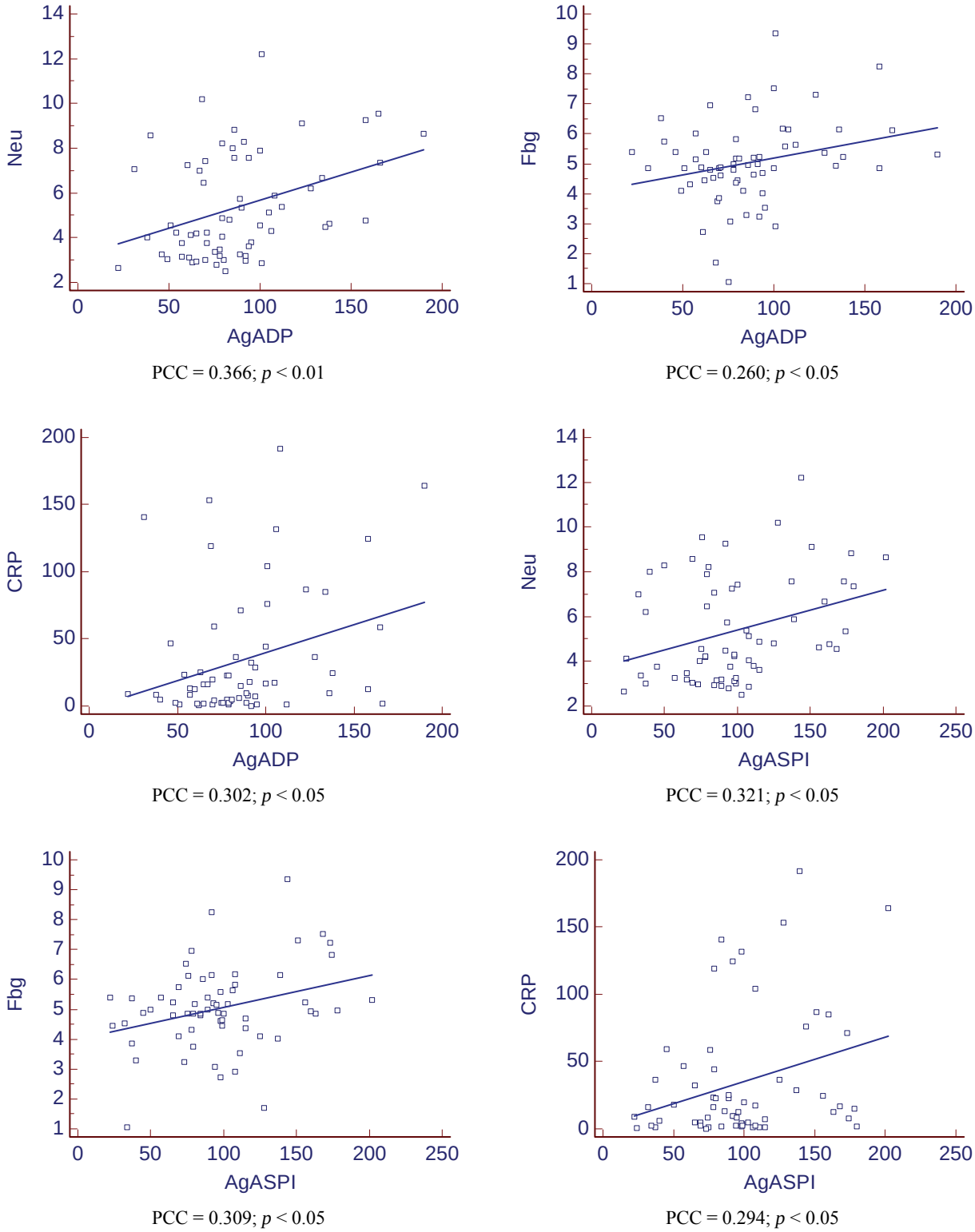

Fig. 1 – Scatter plots showing the corellation between adenosindiphospate (ADP) and arachidonic acid (ASPI) induced platelet agreggation (Ag) and the number of neutrophils (NEU), fibrinogen (Fbg) and C-reactive protein (CRP) in end-stage renal disease (ESRD) patients.
PCC – Perason's correlation coefficient; *p*-value.

Table 2

Values od platelets indicies, platelet aggregation and chronic inflamation and procoagulant activity markers in Group I ESRD and Group II CG, and in subgroup IA DM ESRD and subgroup IB non-DM ESRD

Parameter	Group I ESRD (n = 63)	Group II CG (n = 30)	*p*-value	I A DM ESRD (n = 26)	I B non-DM ESRD (n = 37)	*p**-value
	248.3 ± 110.8	233.6 ± 53.7	ns	277.3 ± 108.9	228 ± 108	ns (*p*=0.08)
NoPlt (× 10^9/L)	3.8 ± 2.3	2.3 ± 1.3	<0.01	3.3 ± 1.9	4.2 ± 2.6	ns
	8.2 ± 1	8.3 ± 0.9	ns	8.1 ± 1.0	8.3 ± 1.1	ns
	0.2 ± 0.1	0.2 ± 0.03	ns	0.2 ± 0.1	0.2 ± 0.1	ns
rPlt (%)	16.6 ± 4	16.3 ± 1.6	ns	16 ± 0.7	17 ± 5.1	ns
MPV (fL)						
PTC (%)						
PDW(fL)						
AgTRAP (%)	115.8 ± 35	98.5 ± 23.4	<0.01	111.0 ± 37	119.2 ± 33.7	ns
AgASPI (%)	98 ± 41.9	82 ± 22.7	<0.05	93.4 ± 45.6	101.3 ± 39.3	ns
AgADP (%)	87.4 ± 34	76.5 ± 18.3	<0.05	85.4 ± 36	88.8 ± 33.0	ns
AgCollagen (%)	65.5 ± 31	51.8 ± 13.3	<0.05	70.6 ± 31.1	62 ± 30.8	ns
aPTT (R)	0.9 ± 0.1	0.9 ± 0.1	ns	0.9 ± 0.1	0.9 ± 0.1	ns
PT (R)	1.03 ± 0.1	1.05 ± 0.1	<0.05	1 ± 0.1	1 ± 0.1	ns
Fbg (g/L)	5 ± 1.4	3.3 ± 0.6	<0.01	5.4 ± 1.1	4.8 ± 1.5	ns
D dimer (ng/mL)	738 (355–1,070.5)	192 (118.3–246.3)	<0.01	748.5 (465–903)	707 (344.8–1,107.5)	ns
vWF Ag (%)	215.8 ± 78.3	142.5 ± 41.6	<0.01	219.4 ± 76.1	213.3 ± 80.7	ns
vWFAct (%)	170 ± 57.9	120.6 ± 36.7	<0.01	174.4 ± 62	167 ± 55.5	ns
	5.4 ± 2.3	3.2 ± 1.1	<0.01	5.8 ± 2.4	5 ± 2.3	ns
NoN (× 10^9/L)	3.2 (2.3–4.9)	1.5 (1.2–1.9)	<0.01	4.1 (2.2–5.3)	3 (2.3–4.3)	ns
	15.1 (3.5–42.4)	0.9 (0.4–1.4)	<0.01	13.7 (4.9–44.4)	16.1 (2.1–39.1)	ns
NLR						
CRP (mg/L)						

The data are expressed as mean ± SD or median (25th,75th percentile); n-sample size; ESRD – end-stage renal disease; CG – control group; DM – diabetes mellitus; non-DM – non diabetes mellitus; *p*-value, difference between Group I ESRD/Group II control group; *p-value, difference between subgroup IA DM ESRD/sub-group IB non-DM ESRD; NoPlt – number of platelets; rPlt – reticulated platelets; MPV – mean platelet volume; PCT – plateletcrit; PDW – platelet distribution width; AgTRAP – thrombin receptor activating peptide aggregation test; AgADP – adenosindiphosphate aggregation test; AgASPI – arachidonic acid aggregation test; AgCollagen – collagen aggregation test; vWF Ag – von Willebrand factor antigen; vWF Act – von Willebrand factor activity; NoN – number of neutrophils; NLR – neutrophil to lymphocyte ratio; CRP – C-reactive protein.**

Discussion

The results indicate several conclusions: first, platelet activity in ESRD is significantly altered and manifested by the increase of reticulated platelets and platelet aggregation; second, the values of inflammatory and procoagulant markers are significantly increased in the ESRD patients compared to the control group of healthy volunteers; third, in ESRD there is a positive correlation between the platelet count, elevated platelet aggregation and values of proinflammatory markers; fourth, the values and correlation of the examined parameters did not differ in sub-groups of patients with regard to the chronic renal failure (CRF) etiological factors; fifth, enhanced reticulated platelets (or augmented platelet turnover) and increased platelet function (or aggregability) in ESRD seem to be more associated with chronic inflammation and procoagulant state rather than with diabetes mellitus as an individual etiological factor of CKD; sixth, neither platelet turnover and function nor inflammatory and procoagulant markers do not predict the likelihood of bleeding in ESRD.

The presence of increased reticulated platelets – "young platelets" with elevated density of granules and RNA content, but similar volume with "mature platelets", is a reliable indicator of enhanced "turnover" or consumption of platelets and increased megakaryocytopoietic activity in the patients with CRF. The above-mentioned fact may be considered important as the absence of thrombocytopenia and lack of changes in platelet volume parameters (MPV, PCT, PDW) in the patients with ESRD compared to the healthy subjects in our study may be explained by the sustained balance between the shortened lifespan and increased level of platelet degranulation (which also represents the indicator of their activation) on one hand, and, on the other hand, the process of sufficiency thrombocytopoiesis i.e. increased "output" of

young platelets as a compensatory mechanism aimed at maintaining homeostasis [13, 14]. Previously published studies of platelet function in ESRD are contradictory and refer to patients undergoing dialysis treatment – haemodialysis or chronic ambulatory peritoneal dialysis [15]. According to them, the measurement of platelet aggregation as the gold standard for testing platelet function using platelet rich plasma (PRP) showed that induced platelet aggregation was either reduced or enhanced. Also, the tests of platelet aggregation using whole blood and determining the platelet activation markers by flow cytometry were inconclusive [16, 17].

In order to avoid the above-mentioned imperfections, we tested platelet functionality in the patients with ESRD who had not yet begun regular dialysis treatment, using the method of whole blood impedance platelet aggregometry which had much better reproducibility [18]. Increased platelet aggregation in our study with conventional inductors (TRAP, ADP and collagen) may be associated with the increased representation of "immature" reticulated platelets subpopulation in the platelet total mass whose haemostatic potential is significantly increased during a very short period of time. This is caused by the presence of mRNA, granular endoplasmic reticulum and ribosomes of megakaryocytic origin and the ability of nucleic synthesis of numerous thrombogenic proteins, glycoprotein platelet membrane, α granule proteins and enzymes, fibrinogen, P-selectin, vWF, GP IIb/IIIa inhibitors and cyclooxygenase-1 (COX-2) [19–21]. Elevated platelet aggregation with arachidonic acid could be further explained by stimulated formation of platelet TxA2 and/or TxA2 generated from the "processing" of excess arachidonic acid (formed as a consequence of endothelial cells damage and decreased binding to albumin in ESRD) by platelet cyclooxygenase-1 (COX-1). This mechanism could "outbalance" the pharmacological effect of possible aspirin use [22–24].

The proportional association between indicators of reduced renal function and increased values of inflammatory and procoagulant markers is already known [25, 26]. Our findings of increased FBG and CRP concentration, NoN, NLR, D dimer, vWF Act and vWF Ag in the patient group are in full conformity with numerous statements regarding the presence of a hypercoagulable state in CKD that occurs due to a complex of disorders of haemostatic balance with reduced fibrinolytic potential and the presence of procoagulant stimulation of multi-causal origin in the background [27, 28].

The endothelial cell damage caused by uremic toxins triggers a complex haemostatic system functionality disorder which affects primary haemostasis and the reactive adaptive response of the body in the form of chronic inflammation and oxidative stress that generates "spreading" of secondary haemostasis functionality disorder, fibrinolytic processes and system of natural inhibitors [29–31].

We considered that a separate study of the relationship of platelet turnover and their functionality with markers of stimulated coagulation activities, especially in the sub-group of patients where DM is the cause of CRF, was justified from the standpoint of the total, additionally negative impact of etiological factors on functionality of all haemostatic system components, scaling up proatherogenic potential and risk for CVD [32].

The theoretical question is the impact of etiological factor as the provoker of kidney disease and CKD *per se* and their dominance in the development of hemostatic system disorders. Some authors state that the risk of venous thromboembolism (VTE) increases with the degree of renal and hemostatic system impairment and that comorbidity has a "modelling" role [33]. It is also known that DM (type-2, type-1) is the most common etiologic factor of CKD in the form of diabetic nephropathy or advanced CKD, as well as combined effect of DM and CKD in the process of atherothrombosis and cardiovascular diseases [34]. In presented results, the influence of etiological factor(s) on the investigated parameters is absent, which can be explained by the small sample size and the lack of differentiation types of diabetes.

It is worth mentioning that distinguishing of the impact of the combined and superimposed metabolic dysfunction, oxidative stress, inflammation and pathological signalling mechanisms on the molecular base of prothrombotic state was beyond our everyday capabilities and in practice routinely used clinical – laboratory diagnostic procedures and methods [35–37].

Limitations of this study include a small number of respondents determined by the CKD incidence in our general population, the fact that it was observational and that it did not exclude the impacts of confounding factors (smoking, hypertension, hyperlipidemia, cardiovascular comorbidities and the use of various drugs) in the ESRD patients [38].

Conclusion

Results and correlation of the studied parameters point out increased platelet turnover in ESRD as a consequence of platelet activation and consumption induced by clotting system hyperactivity and chronic inflammation i.e. prothrombotic state and that it was nonsignificantly influenced by etiological factors. The presence of increased reticulated platelets is a reliable sign of their increased consumption and preserved megakaryocytopoiesis in ESRD. Although none of the examined parameters did not indicate the likelihood of bleeding in ESRD, in everyday clinical practice in the absence of more precise method, both whole blood platelet aggregation (indicator of platelet functionality) and reticulated platelets (indicator of increased platelet turnover) could be used as auxiliary laboratory methods in timely identification of patients with higher susceptibility to the occurrence of one of the two extremes, clinically "opposite" and therapeutically completely differently managed disorders of hemostatic system functionality in CRD – bleeding or thrombosis. Within this statement, the need for further testing of rational use of antiplatelet therapy in CRF in prevention of CVD is also imposed.

Conflict of interest

The authors reports no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Psychological and spiritual well-being aspects of the quality of life in colostomy patients

Psihološki i duhovni aspekti kvaliteta života bolesnika sa kolostomom

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Abstract

Background/Aim. Colorectal cancer and its treatment can have a negative impact on the quality of life which has become an important outcome measure for cancer patients. The aim of this work was assessment of psychological and spiritual dimension of the quality of life in colostomy patients, regarding the gender and age. **Methods.** This is a cross-sectional study conducted at the Abdominal Surgery Polyclinic in the Clinical Canter of Vojvodina among colostomy patients operated between January 2010 and June 2011. The instrument used in this study was Quality of Life Questionnaire for a Patient with an Ostomy (QOL-O). **Results.** Majority of respondents were male (M:F = 50.7% : 49.3%). The age ranged between 36–86 years. Respondents did not report difficulties in adjustment to stoma, but their great difficulty was to look at it and the sense of depression and anxiety. The care of stoma was worse perceived by younger respondents ($p = 0.014$). Respondents were mostly satisfied with their memorizing ability and having the sense of control. The lowest score was found in sensing satisfaction or enjoyment in life. The age had a significant impact on positive aspects of psychological well-being ($p < 0.05$). Higher scores were found among younger age groups. The mean score of spiritual well-being (6.47 ± 3.01) was lower than the mean score of psychological well-being (7.76 ± 2.35). There were no statistically significant differences regarding gender (t = -0.738, df = 65, $p = 0.463$) or age (F = 1.307, $p = 0.280$). **Conclusion.** Psychological and spiritual well-being in colostomy patients appeared to be at satisfactory level, but it is necessary to provide tailor made support in order to prevent and resolve negative responses to stoma.

Key words:
colostomy; adaptation, psychological; spirituality; surveys and questionnaries; treatment outcome.

Apstrakt

Uvod/Cilj: Kolorektalni karcinom negativno utiče na kvalitet života obolelih i važan je parametar ishoda lečenja bolesnika sa dijagnozom malignih bolesti. Cilj istraživanja bio je procena psiholoških i duhovnih aspekata kvaliteta života bolesnika sa kolostomom u odnosu na njihov pol i starost. **Metode.** Istraživanjem je bilo obuhvaćeno 67 bolesnika oba pola, koji su nakon operativnog zahvata na kolonu sa izvedenom kolostomom, ambulantno praćeni u Specijalističkoj poliklinici Kliničkog centra Vojvodine. Za potrebe istraživanja korišćen je Upitnik za procenu kvaliteta života pacijenata sa kolostomom [*Quality of Life Questionnaire for a Patient with an Ostomy* (QOL-O)]. **Rezultati.** Starost ispitanika iznosila je 36–86 godina. Većinu ispitanika činili su muškarci (50,7%). Većina ispitanika nije imala poteškoća kod adaptacije na stomu; najteže im je bilo da gledaju stomu, a imali su i osećaj depresije i anksioznosti. Mlađim ispitanicima je bilo teže da neguju svoju stomu ($p < 0.05$). Ispitanici su uglavnom bili zadovoljni sposobnošću pamćenja i osećanjem kontrole. Najviše ocene kod pozitivnog aspekta psihološke dimenzije kvaliteta života uočene su u mlađim dobnim grupama ($p < 0.05$). Prosečna ocena spiritualne dimenzije kvaliteta života (6,47 ± 3,01) bila je niža u odnosu na prosečnu ocenu psihološke komponente (7,76 ± 2,35), bez značajnih razlika u odnosu na pol (t = -0.738, df = 65, $p = 0.463$) ili starost (F = 1.307, $p = 0.280$). **Zaključak.** Mada su samoprocenom psihološke i duhovne komponente kvaliteta života ispitanika dobijeni zadovoljavajući rezultati, neophodno je obezbediti specifičnu podršku u cilju prevencije i otklanjanja negativnih reakcija na stomu bolesnika sa kolostomom.

Ključne reči:
kolostomija; adaptacija, psihološka; duhovnost; ankete i upitnici; lečenje, ishod.

Introduction

Colorectal cancer is the second most prevalent cancer in the Province of Vojvodina (northern region of the Reublic of Serbia) observing the rates of incidence and mortality. During 2007 about 1233 new cases (12.76 % of all cancer cases) and 778 deaths (12.69% of all deaths) were registered in Vojvodina. As in other regions worldwide, incidence and mortality rates are higher in men than in women (sex ratio 1.5 : 1). Age-specific incidence and mortality rates are incre-

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asing rapidly from the age of 50, with the highest level in the age group of 75–79 [1].

In Serbia, the standardized incidence rate for colorectal cancer is 27/100,000, 33.5 for male (M) and 21.6 for female (F), respectively. The incidence rates rise with the age, and they are at their highest at the age of 70–74 in men and 75 and more in women when considering the sex. In other countries as well as in Serbia, there is a growing evidence of higher incidence in population younger than 40 years. Colorectal cancer is the second leading cause of death in male and the third one in female population in Serbia. According to the standardized mortality rates, Serbia can be referred to the category of countries with high mortality rates. Highest mortality rates can be found at the age of 75 and older, both in men and women [2].

According to reversibility, stoma can be divided into a temporary or a permanent one [3]. Formation of stoma can significantly influence the general health status of a patient, in particular his/her psychosocial adaptation process after the surgery. Stoma patients become unable to control defecation and presence of stoma on the abdominal surface radically changes the image of one's body [4]. Patients submitted to colostomy have to face two major issues: the cancer, a disease that carries the stigma of death and suffering and the stoma, a physical mutilation, which, despite the fact that is kept hidden, brings many consequences. A stoma patient faces the loss of sphincter control caused by the opening of an intestinal stoma and is concerned about the odor, leakage and physical discomfort, which can be a factor that affects interpersonal relationships [5].

Since creation of the stoma seems to be a great stressor for a patient, early rehabilitation is necessary to be proceeded immediately after the surgery. This should include training for patients for self-care and self-help. Indeed, all rehabilitation activities should be aimed at re integration in everyday activities. The rehabilitation process has physical and psychological component – solving adaptation issues [6].

The World Health Organization defines the quality of life as "an individual's perception of their place in life in the context of culture and value systems in which they live and in relation to their goals, expectations, standards and concerns" [7]. The term *quality of life* (QOL) refers to a multidimensional concept, which includes, at least, the dimensions of physical, emotional, and social functioning. Therefore, besides traditional indicators, like disease-free and overall survival time, the QOL has become an important outcome measure for cancer patients. The aassessment of QOL in patients with cancer may improve our understanding of how cancer and therapy influence the patients' lives and how to adapt treatment strategies [8].

Colostomy patients, despite fighting the cancer, have poor body image and self esteem, and experience anxiety of rejection. The awareness of the change in the physical body and suffering regarding the new lifestyle affect the physical and psychological aspects, as well as social relationships and environment, compromising the quality of life in whole. In certain cases stoma creation can lead to suicide attempts, due to social isolation, anxiety and depression. Some changes in social roles are also inevitable. Therefore stoma patients have to adjust to a number of changes that he/she perceive as negative [6, 9].

The aim of this study was an assessment of psychological and spiritual aspects of the quality of life in colostomy patients regarding the gender and the age.

Methods

Sampling design

This survey was designed as a cross-sectional study. The main criterion for inclusion in the survey was elective stoma creation between January 2010 and June 2011 and regular follow-ups at Specialistic Polyclinic in the Clinical Center of Vojvodina as well as adult age, having adequate physical and mental health and willingness to participate in the study.

All of the patients who fulfilled these criteria were asked to participate in this survey. Critera for exclusion were unwillingness to participate, insufficient knowledge of Serbian language and mental health issues.

Patients were asked to complete a questionnaire while undergoing regular check-ups. The questionnaires were administered directly to patients by the investigators who facilitated answering the questions while clarifying doubts and difficulties. Patients were informed of the purpose of the study and provided written consents. Participation in the survey was voluntary. Anonymity was ensured. The questionnaires were collected immediately after they were completed. The entire procedure took 15–20 minutes to complete.

Respondents

The total number of 86 questionnaires were distributed, but 67 of them were returned with positive response regarding participation in the study. The response rate was 77.90%.

Out of 67 participants, 49.3% were females and 50.7% were males. The mean age was 65.87 [standard deviation (SD) = 10.16] years and it ranged from 36 to 86 years. The majority of patients (44.8%) were 70 years old or older. As far as their educational level was concerned, more than half (56.7%) of the patients completed high school while one quarter (25.4%) of them completed elementary school.

Three types of stoma were observed among respondents. There were 47 (70.14%) patients with colostomies, 11 (16.41%) patients with ileostomies, and 9 (13.45%) patients with urostomies. Two-thirds of the patients had permanent colostomy. Majority (90.6%) of stomas were due to the malignant disease.

Time after surgery appeared to be an important parameter regarding the quality of life, because it takes time to accept the changes and adjust to life with stoma, re-socialize and return to prior activities. About two-thirds of the patients underwent the surgery up to 12 months prior to survey, while 37.0% had surgery between 12–24 months prior to this survey. The mean time elapsed from the surgery was 11.52 (SD = 5.06) months (2–24 months).

Instrument

Participants' age, gender, socioeconomic status, type of colostomy and time elapsed after stoma creations were included in the demographic features.

The instrument used in this study to assess the quality of life of patients with colostomy was the Quality of Life Questionnaire for a Patient with an Ostomy (QOL-O) designed by Grant et al. [10]. The questionnaire had two components. The first component consisted of 47 forced-choices and open ended items that relate to patient sociodemographic characteristics as well as work-related items, health insurance, sexual activity, psychological support, clothing, diet, and daily colostomy care. The second component contained 43 QOL items using 10-point scales. These QOL items were divided into four domains or subscales: physical well-being, psychological well-being, social well-being and spiritual well-being. The respondents were asked to assess every item using one of suggested marks, where 0 was the worst outcome/negative QOL and 10 was the best outcome/positive QOL. Some items in the domain of psychological and spiritual well-being which we present in this paper had to be reversing coded prior to data entry. Subscale scores were produced by adding the scores on each item with the subscale and then dividing by the number of items in that subscale.

The study was approved by the Ethics Committee of the School of Medicine in Novi Sad.

Statistics

Survey data were analysed in SPSS 18.0. Statistical analysis included descriptive and inferential analysis. Descriptive analysis included the total value expressed in absolute and relative numbers. The *t*-test and one-way ANOVA were used to test for difference between subgroups. Statistically significant values were considered to be at the level of $p < 0.05$.

Results

Psychological aspect of quality of life

Psychological well-being was assessed through 13 items. Some negative aspects, such as difficulties in adjustment to stoma, embarrassment, difficulties to look at their stoma, difficulties in self-care, and having emotions like anxiety, depression and fear were assessed in this domain. There was one missing answer for questions regarding embarrassment for having colostomy (Table 1).

Table 1

Psychological well-being

Item	n	Mean	SD	Min	Max
Negative aspects					
How difficult has it been for you to adjust to your colostomy?	67	2,07	3.25	0	10
How much are you embarrassed by your colostomy?	66	9.55	1.96	0	10
How difficult is it to look at your colostomy?	67	9.66	1.55	1	10
How difficult is it for you to care for your colostomy?	67	9.01	2.00	0	10
How much anxiety do you have?	67	9.43	1.17	5	10
How much depression do you have?	67	9.58	1.62	0	10
Are you fearful that your disease will come back?	67	8.88	1.25	2	10
Positive aspects					
How useful do you feel?	67	8.79	1.66	1	10
How much satisfaction or enjoyment in life do you feel?	66	8.50	2.21	0	10
How good is your overall quality of life?	66	8.53	1.99	0	10
What is your ability to remember things?	67	9.28	1.50	0	10
Do you feel like you are in control of things in your life?	67	8.90	2.32	0	10
How satisfied are you with your appearance?	67	8.87	1.17	1	10

n – number of respondents; SD – standard deviation; min – minimal value; max – maximal value.

Positive aspects of physical well-being of colostomy patients were assessed through items that covered life satisfaction, memory ability, and sense of control and evaluation of self-image. Respondents were mostly satisfied with their ability to remember things and having sense of control. The lowest score was found in having sense of satisfaction or enjoyment in life. In two items (How much satisfaction or enjoyment in life do you feel?; How good is your overall quality of life?), one missing answer was found (Table 1).

When assessing the gender differences in negative aspects of psychological well-being, it was observed that there were no statistically significant differences among male and

female respondents. It was obvious that self-care of stoma was worse participated by respondents in the younger age groups (p = 0.014), while in other items there were no significant differences regarding age (Table 2).

Gender differences were not statistically significant while observing items considering the positive aspects of physical well-being (Table 3). On the other hand, it was observed that higher scores were more frequent among younger age groups compared to older respondents (Table 3).

The total score of this domain was produced by adding scores of all domain items and dividing the sum by number of items. The score for the domain of psychological well-being was 7.76 (SD = ± 2.35), widely ranging from 0.80–9.60. No statistically significant differences among gender (t = -0.584, df = 35, p = 0.563) or age groups (F = 2.205, p = 0.106) were found.

Table 2

Gender and age differences in negative aspects of psychological well-being

Item	Gender, mean ± SD		Age (years), mean ± SD				
	male/ female	p	≤ 49	50–59	60–69	≥ 70	p
Adjustment to stoma	1.60 ± 2.77/ 2.59 ± 3.69	0.214	1.00 ± 1.22	3.00 ± 4.34	2.10 ± 3.65	1.90 ± 2.77	0.868
Embarrassment	9.44 ± 2.15/ 9.66 ± 1.77	0.660	10.00 ± 0.00	9.09 ± 3.22	9.95 ± 0.22	9.23 ± 0.55	0.819
Looking at stoma	9.40 ± 2.12/ 9.94 ± 0.25	0.159	10.00 ± 0.00	10.00 ± 0.00	9.90 ± 0.30	9.30 ± 2.28	0.415
Self-care of stoma	9.00 ± 1.78/ 9.03 ± 2.24	0.950	10.00 ± 0.00	9.91 ± 0.30	9.52 ± 1.17	8.17 ± 2.59	0.014*
Anxiety	9.40 ± 1.09/ 9.47 ± 1.27	0.812	9.00 ± 2.24	9.55 ± 0.93	9.48 ± 1.17	9.43 ± 1.07	0.852
Depression	9.63 ± 1.26/ 9.53 ± 1.95	0.808	10.00 ± 0.00	9.00 ± 3.00	9.67 ± 1.53	9.67 ± 1.03	0.603
Fear	8.86 ± 1.03/ 8.91 ± 1.47	0.874	040 ± 0.89	9.36 ± 0.50	9.00 ± 1.50	8.53 ± 1.50	0.170

SD – standard deviation; $p < 0.05$*

Table 3

Gender and age differences in positive aspects of psychological well-being

Item	Gender, mean ± SD		Age (years), mean ± SD				
	male/ female	p	≤ 49	50–59	60–69	≥ 70	p
Feeling of usefulness	8.83 ± 1.38/ 8.75 ± 1.93	0.848	9.20 ± 1.30	9.82 ± 0.40	9.24 ± 1.61	8.03 ± 1.73	0.004**
Satisfaction in life	8.59 ± 1.89/ 8.41 ± 2.53	0.741	8.80 ± 1.30	9.91 ± 0.30	9.24 ± 1.26	7.38 ± 2.72	0.001***
Good quality of life	8.37 ± 2.20/ 8.71 ± 1.75	0.496	9.40 ± 0.89	9.60 ± 0.70	9.29 ± 1.06	7.50 ± 2.42	0.001***
Remembering ability	9.26 ± 1.72/ 9.31 ± 1.23	0.881	9.20 ± 1.79	9.91 ± 0.30	9.67 ± 0.66	8.80 ± 1.95	0.088
Sense of being in control	8.69 ± 2.42/ 9.13 ± 2.23	0.444	10.00 ± 0.00	9.91 ± 0.30	9.52 ± 1.17	7.90 ± 3.07	0.013*
Satisfaction with appearance	8.49 ± 2.38/ 9.28 ± 1.28	0.097	9.40 ± 0.89	9.82 ± 0.40	9.52 ± 1.17	7.97 ± 2.47	0.008*

SD – standard deviation; p < 0.05*; $p < 0.05$; $p < 0.05$*****

Spiritual aspect of quality of life

Last domain in questionnaire had seven items regarding spiritual dimension of the quality of life. The mean score of negative aspect in the domain of spiritual well-being was 8.12 (SD = ± 2.97). Participants in the younger age groups showed greater level of insecurity, comparing to the older respondents (F = 6.056, p = 0.001), while differences between gender were not at statistically significant level (t = -1.086, df = 65, p = 0.281).

Positive aspect in the domain of spiritual well-being was represented with six items. One missing answer was found for two items ("How hopeful do you feel?" and "Is support you receive from personal spiritual activities such as prayer or mediation sufficient to meet your needs?"). Nine missing answers were found in the item reading as "Is support you receive from religious activities such as going to church or synagogue sufficient to meet your needs?", while item "Has having a colostomy made positive changes in your life style?" had the lowest response rate (41.8%). The best scores were found in a statement about having a sense, a reason of being alive and having a hope, while a very small number of respondents perceived positive impacts of stoma on their life (Table 4).

In this study no statistically significant differences between males and females, regarding items of spiritual well-being were found, while number of respondents who saw positive changes after having colostomy significantly decreased in older age groups ($p = 0.021$) (Table 5).

The total score of this domain was lower than in the domain of psychological well-being (6.47/SD = ± 3.01), ranging from 0.00–9.60. No statistically significant differences between gender (t = -0.738, df = 65, $p = 0.463$) or among age groups (F = 1.307, $p = 0.280$) were found.

Table 4

Spiritual well-being

Item	n	Mean	SD	Min	Max
Do you sense a reason for being alive?	67	9.13	2.08	0	10
Do you have a sense of inner peace?	67	9.46	1.51	0	10
How hopeful do you feel?	66	9.21	2.03	0	10
Is support you receive from personal spiritual activities such as prayer or mediation sufficient to meet your needs?	66	6.26	3.62	0	10
Is support you receive from religious activities such as going to church or synagogue sufficient to meet your needs?	58	5.48	3.52	0	10
Has having a colostomy made positive changes in your life style?	28	3.93	3.94	0	10

n – number of respondents; SD – standard deviation; min – minimal value; max – maximal value.

Table 5

Gender and age differences in spiritual well-being

Item	Gender, mean ± SD		Age (years), mean ± SD				
	male/ female	*p*	≤ 49	50–59	60–69	≥ 70	*p*
Sense of reason for being alive	8.77 ± 2.62/ 9.53 ± 1.16	0.137	9.00 ± 0.71	9.82 ± 0.60	9.57 ± 1.16	8.60 ± 2.85	0.252
Sense of inner peace	9.31 ± 1.98/ 9.63 ± 0.71	0.405	9.20 ± 1.10	10.00 ± 0.00	9.76 ± 0.70	9.10 ± 2.09	0.254
Feeling of hopefulness	9.21 ± 1.98/ 9.22 ± 2.11	0.980	8.00 ± 4.47	9.82 ± 0.40	9.38 ± 2.18	9.07 ± 1.67	0.389
Spiritual activities	5.88 ± 3.73/ 6.66 ± 3.52	0.390	6.00 ± 4.69	5.45 ± 4.03	4.67 ± 3.89	7.76 ± 2.49	0.019*
Religious activities	5.14 ± 3.48/ 5.83 ± 3.58	0.460	3.33 ± 4.16	4.55 ± 4.03	4.44 ± 3.50	6.85 ± 2.88	0.056
Positive changes in life due to colostomy	4.13 ± 3.67/ 3.83 ± 4.25	0.767	8.25 ± 0.96	1.86 ± 3.29	1.25 ± 2.50	4.54 ± 4.03	0.021*

SD – standard deviation; $p < 0.05$*

Discussion

There is growing evidence about rise in prevalence of colorectal cancer worldwide [11], so it can be expected that the number of patients with stoma will increase as well. Therefore, issues on the quality of life of stoma patients have to be evaluated more closely.

Ever since WHO gave the definition of the quality of life in 1948, many instruments for measuring individual well-being were developed. The assessment of the quality of life has been explored in many studies, where correlation between functional and psychological issues had been assessed [12].

In this study we evaluated two domains of the quality of life - psychological and spiritual. As a consequence of physical function and altered body appearance, some psychological issues may arise. Stoma creation causes profound changes because of physical deterioration, loss of bodily function, changes in daily routines and restriction in level of social functioning. Such changes have negative impacts on psychological well being [13].

In our study, in the domain of psychological well-being, some negative and positive aspects were assessed. As of negative psychological issues that stoma patients might experience, it was observed that they did not report difficulties in adjustment to stoma, but their great difficulty was to look at it, i.e. the sense of depression and anxiety Danielsen [14] referred to studies she reviewed and stated that depression, loneliness, suicidal thoughts and low self-esteem were significantly more prevalent in patients with stoma, compared to patients without stoma. Different results were found in a study performed by Krousse et al. [15] who came to a conclusion that both cancer and non-cancer stoma patients, did not report problems with looking at their own stoma.

Sharpe et al. [16] in their study explored relationship between body image disturbance and distress in colorectal cancer patients with and without stomas. They found out that

body image was a strong predictor of initial levels of anxiety, depression, and distress and subsequent anxiety and distress.

Positive aspect of psychological well-being showed satisfaction of respondents, mostly with their ability to remember things and having sense of control, while the lowest score was found in having sense of satisfaction or enjoyment in life. No statistically significant differences between gender groups were found. On the other hand, age had significant impact and for this reason the higher scores were more frequent among the younger age groups, compared to the older respondents in five out of six items (feeling of usefulness, satisfaction in life, good quality of life, sense of being in control and satisfaction with appearance).

In this study, gender differences in the domain of psychological well-being were not at significant level, although, regarding negative aspect of physical well-being, we found that self-care of stoma was worse perceived by respondents in younger age groups. Arndt et al. [17] discussed that younger colorectal patients might express psychosocial deficits, probably because they have fewer coping strategies for managing a life-threatening disease, comparing to their older peers. Therefore, results in their study indicated that younger colorectal cancer patients suffer from deficits in emotional and social functioning.

Liao and Qin [18] stated that sense of hope is a significant motivating and coping factor. Referring to the reviewed literature, they stated that it was demonstrated in several studies that hope can mediate a variety of psychological effects on QOL in cancer patients.

Dabirian et al. [19] conducted a qualitative study about quality of life of stoma patients in Iran on 14 subjects aged 14–57 years, of different gender and personal background, who had permanent colostomy. Some cognitive and mental issues aroused and they are fear of impact of disease on family members. They emphasised that being a member of support group would improve their self-esteem.

In the domain of spiritual well-being, the total of seven questions were offered in questionnaire - one about negative (How much uncertainty do you feel about your future?) and other six about positive aspects of spiritual well-being (Do you sense a reason for being alive?; Do you have a sense of inner peace?; How hopeful do you feel?; Is support you receive from personal spiritual activities such as prayer or mediation sufficient to meet your needs?; Is support you receive from religious activities such as going to church or synagogue sufficient to meet your needs?; Has having a colostomy made positive changes in your life style?). Regarding the sense of insecurity about one's future, it was observed that participants in the younger age groups showed greater level of insecurity, comparing to their older peers maybe because older persons perceive their physical status in a difference frame, comparing to the younger age groups [20].

When it comes to positive aspect in the domain of spiritual well-being, it is encouraging that the best scores were found in questions about having a sense of inner peace, a reason of being alive and having a hope, while a very small number of respondents perceived positive impact of stoma on their life; that was an item with both lowest score and response rate. Gender and age did not have significant impact on distribution of answers, except whether the colostomy made positive changes in ones life style; number of respondents who saw positive changes after having colostomy significantly decreased in the older age groups.

It was shown in our study that fewer respondents were engaged in religious and spiritual activities. Respondents in the older age groups received support from personal spiritual activities such as prayer or mediation more frequently than the younger respondents. On the contrary, respondents in a study performed by Dabirian et al. [19] found that a spiritual dimension and religious rituals were of great importance to them (respondents had Islamic religion). Kimura et al. [5] referred that religion can bring relief to suffering since the spiritual well-being is associated with psychological dimension as well as with cultural background.

Conclusion

Although self-assessment of psychological and spiritual well-being in colostomy patients gave satisfactory results, providing continuous support by health and social services for a stoma patient and his/her family is necessary in prevention and management of negative reactions toward stoma and improvement of their quality of life.

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Inequalities in health in a municipality of Serbia

Nejednakost u zdravlju na području jedne opštine u Srbiji

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Abstract

Background/Aim. A consistent association between socioeconomic determinants and health related variables has been found in many European countries. The aims of this study were: to analyze the association of socioeconomic factors with self-perceived health and utilization of health services as well as to suggest some interventions to overcome the existing problems. **Methods.** Hybrid study was peformed. The two cross-sectional studies were conducted on quota samples (1999 and 2015) in Kruševac Municipality. The questionnaire was used as the investigation instrument for 196 interviewees in 1999 and 226 interviewees in 2015. **Results.** In the reporting period, there were the following results: a significant increase in people who did not have a steady income (χ^2 = 22.800; df = 4; p < 0.01), a decrease in the number of people who perceived their own health as "well" and "very well", a significant increase (6.1%) in people who did not visit anyone when disease occurred, a decrease of 13.2% in number of people who, at least once, visited the general practitioner and an increase in the number of people who visited private health care sector. The findings revealed inequalities in self-perceived health depending on socioeconomic position, in particular educational and employment status (χ^2 = 11.293; df = 4; p < 0.05). There are two major ways in which unemployment affects health: lack of income and ability to meet daily needs and emotional stress related to the meaning of the work, uncertain future, loss of self-esteem, and identity. **Conclusion.** Equality is a key value in the assessment of the effects on health. It is necessary to conduct effective interventions for overcoming the consequences in society that would be focused on a specific target group in one territory.

Key words:
health status; socioeconomic factors; health services; serbia.

Astrakt

Uvod/Cilj. U mnogim zemljama Evrope opisana je postojana povezanost između socijalno-ekonomskih faktora i zdravlja. Ciljevi ovog istraživanja bili su da se analizira povezanost između socijalno-ekonomskih faktora i samoprecene zdravstvenog stanja kao i korišćenja zdravstvenih usluga, i da se sagledaju intervencije za prevazilaženje uočenih problema. **Metode.** Sprovedena je hibridna studija. Dve uzasopne studije preseka (1999. i 2015. godine) su realizovane na uzorku stanovnika (196 ispitanika u 1999. i 226 ispitanika u 2015. godini) gradskog jezgra grada Kruševca. Instrument istraživanja je bio upitnik. **Rezultati.** U posmatranom periodu došlo je do: značajnog povećanja udela onih koji nemaju stalne izvore prihoda (χ^2 = 22.800; df = 4; p < 0.01), smanjenja udela onih koji svoje zdravlje percipiraju kao dobro i izuzetno dobro, značajnog povećanja (za 6,1%) onih koji se u slučaju bolesti ne javljaju nikome i smanjenja broja za 13,2% onih koji su potražili pomoć lekara barem jedanput, povećanja udela onih koji se obraćaju lekaru u privatnom sektoru zdravstva. Rezultati ukazuju na nejednakost u samoproceni zdravlja u odnosu na socijalno-ekonomski položaj, a posebno u odnosu na nivo obrazovanja i status zaposlenosti (χ^2 = 11.293; df = 4; p < 0.05). Nezaposlenost na dva načina utiče na zdravlje: preko nedostatka materijalnih sredstava i preko nedostatka sposobnosti da se zadovolje dnevne potrebe, kao i preko emocionalnog stresa povezanog sa gubitkom posla, neizvesnom budućnosti, gubitkom samopouzdanja i identiteta. **Zaključak.** Jednakost je ključna vrednost u proceni uticaja na zdravlje. Neophodne su efektivne intervencije za prevazilaženje posledica nejednakosti u društvu, koje bi se sprovele na određenoj ciljnoj grupi na jednom području.

Ključne reči:
zdravstveno stanje; socijalno-ekonomski faktori; zdravstvene službe; srbija.

Introduction

Inequalities in health of both an individual and population are inevitable. They come as consequences of a difference in genes, social and economic living conditions or they are a result of an individual's choices and actions. Also, the inequalities in health come as a consequence of a difference in possibilities of individuals (inequality in healthcare acces-

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sibility, housing differences, healthy eating or physical activity). Poverty is one of the main causes of health degradation.

In the year 1820, the ratio between the rich and the poor were 3 : 1 and in 1992, it was 72 : 1 [1]. The countries with developed democracies have 86% of gross domestic product [1].

Equity in health issues involves trying to understand and give people what they need to enjoy full and healthy lives. Equality, in contrast, aims to ensure that everyone gets the same things in order to enjoy full and healthy lives. Like equity, equality aims to promote fairness and justice, but it can only work if everyone starts from the same place and needs the same things [2]. As the Pan-American Health Organization puts it, equity is the means; equality is the outcome [2]. Equity means social justice or fairness. It is an ethical concept, grounded in principles of distributive justice [3]. Equity in health care is defined as the absence of systemic disparities in health (or in the major social determinants of health) between social groups who have different levels of underlying social advantages/disadvantages, that is, different positions in a social hierarchy [3]. Inequities in health systematically put groups of people who are already socially disadvantaged (for example, by being poor, female, and/or belonging to some disenfranchised racial, ethnic or religious group) even in a worse position with respect to their health [3]. Equity in health care is defined as: equal access to available care for equal need, equal utilization for equal need and equal quality of care for all [4]. Financial, organizational and cultural barriers confront people wanting to use services, so, although they may have right to health care in theory, their access may be restricted in practice. Inequities in access also arise when resources and facilities are unevenly distributed around the country, clustered in urban and more prosperous areas and scarce in deprived and rural neighborhoods [4].

Equality and equity mean that the full attention must be diverted to those who need it most, to those who are carrying the greatest burden of illness and to those who are receiving inadequate medical attention or are endangered by poverty. Moreover, one of the principles of the healthcare policies is reduction of inequalities in health.

Today, image of Serbia is characterized by a socially stratified society, with an ever more drastic inequality between social layers. Social differences are more and more pronounced in Serbia, which have been verified by experts. The last research of this phenomenon in Serbia showed that the inequality coefficient in the year 2013 was 38, which means that the country is slipping towards the zone of a pronounced inequality [5] (until 2009 researches with the Gini coefficient for measuring the economic inequality was 28 which showed that Serbia was not in this zone). Serbia, along with Macedonia is the country with the highest level of economic inequality in Europe [5].

Although there is available data that the number of poor people in Serbia decreased, a part of them, who were in a multiply unfavorable position when it comes to education, healthcare and living standards, represented 3.1% of the population become poorer [6]. The intensity of their poverty worsen from 38.3% in 2005 to about 40% in 2010 [6]. The average growth of social development in Serbia (0.34%) between 2000 and 2013, was the lowest in the region [6], and a drop was predicted in the upcoming period due to the combination of a drop in income and difficulties in accessing education and healthcare [6].

The great inequalities contribute, on one side, to socially disintegrative processes and, on the other one, they make a county's exit out of poverty more difficult. Demographic changes are closely related to the issues of inequality and poverty, aging of the population, decrease in the cultural influence on the development of a country as well as the increase in pathological disorders in the society.

Many researches have shown a mutual directly proportional relationship between health and income [7–9]. Furthermore, it was concluded that life expectancy and education had a direct and indirect (over income) impact on health[7]. Thus, socioeconomic variables (such as income, education, profession, employment) have the same, or just a little less, effect on the health as well as lifestyle [10, 11].

The main reason for inequality in the city of Kuševac in Serbia is a high unemployment rate, since the data acquired by the National Employment Agency show that the rate is higher than in the country (around 36% in 2013, as opposed to 25% in Serbia) [12].

In the previously described conditions, it is important for public health professionals to perceive the size of the challenges as consequences of inequality and to discover ways to reduce the impacts of this inequality on the health of the inhabitants in a certain region in a sustainable and adequate manner.

Methods

The method used in this research belongs to the group of analytical epidemiological methods called hybrid studies, in one of their subgroups, that is, repeated measures designed study. Two consecutive cross-sectional studies were undertaken, in September and October of 1999 and in September of 2015. The research was conducted on a 5‰ quota sample of the population of the city of Kuševac (inhabitants older than 18). In this way, 196 participants were surveyed in the year 1999 and 226 participants in 2015. The analysis of sociodemographic characteristics of the participants showed that a somewhat larger amount of women than men was surveyed (53.1%), the largest number of participants had only high school education (55.3%), while 20.4% of the participants had some form of higher education and 28.2% of the participants only graduated from elementary school or completed apprenticeship in some craft. As there were no statistically significant differences in the distribution by gender ($\chi^2 = 0.00454$, $df = 1$, $r > 0.05$), age ($\chi^2 = 0.043$, $df = 2$, $r > 0.05$) and educational attainment ($\chi^2 = 0.019$, $df = 2$, $r > 0.05$) between the population who filled out the questionnaire and those in census, a sample of the population of Kruševac can be considered representative, so, the results of the research can be generalized to the entire population of Kruševac.

The instrument was a questionnaire with 20 questions divided into four sections: demographic and socioeconomic

variables (8 questions), self-assessed health (5 questions), the use of healthcare (5 questions) and out-of-pocket paying for health services (2 questions). Information was gathered through "face to face" interviews. The participants with previously determined characteristics were surveyed in their houses and workplaces.

The data were processed using the methods of descriptive statistics and their relevance was tested with χ^2-test.

Results

As far as the percentage of participants who had a stable income (full time employment of pension) in 1999 (83.2%) and 2015 (63.7%) was considered, there were significant changes as well as in the pool of the participants with part- time employment or unemployed or "housewives" (10.2%, and 23.6%, respectively) (Table 1). This difference was highly statistically significant ($\chi^2 = 22.800$; df = 4; $p < 0.01$).

Table 1

The distribution of respodents employment status in two observed periods (1999 and 2015)

Employment	1999	2015	Total
	n (%)	n (%)	n (%)
A steady job and a pensioner	163 (83.2)	144 (63.7)	307 (72.7)
Occasional	9 (4.6)	38 (16.8)	47 (11.2)
Unemployed and housewives	20 (10.2)	37 (16.4)	57 (13.5)
On education, etc.	4 (2.0)	7 (3.1)	11 (2.6)
Total	196 (100)	226 (100)	422 (100)

$\chi^2 = 22.8$; df = 3; $p < 0.01$.

The average income per capita in surveyed households in 2015 was 9,055.96 dinars (€75), with mod as typical value as 10.000 dinars (€83) and in those who were surveyed in 1999 it was 1.078,00 dinars (€45), with mode of 1,000 dinars (€42). Although respondents in 2015 their material status generally described as better than in 1999, the differences in frequency were not statistically significant ($\chi^2 = 4.601$; df = 2; $p > 0.05$).

In 1999, 53.1% of the surveyed participants assessed their health as good or very good, while in 2015 this percentage was smaller – 45.1%, but the differences in frequency were not statistically significant ($\chi^2 = 3.503$, df = 2, $p > 0.05$) (Table 2).

Table 2

Distribution of answers on the self-assessment of health status of respondents in in two observed period (1999 and 2015)

Self-assessment of health status	1999	2015	Total
	n (%)	n (%)	n (%)
Extremely well and good	104 (53.1)	102 (45.1)	206 (48.8)
Moderately	76 (38.8)	96 (42.5)	172 (40.8)
Bad and pretty bad	16 (8.1)	28 (12.4)	44 (10.4)
Total	196 (100)	226 (100)	422 (100)

$\chi^2 = 3.503$; df = 2; $p > 0.05$.

A statistically significant relationship between self-assessed health and material status of the participant was established ($\chi^2 = 11.293$; df = 4; $p < 0.05$) in a sense that the participants who assessed their material status as very good or good were more prone to assess their health as good or very good, and the ones who assessed their material status as bad and very bad, assessed their health in the same manner, too. The situation remained unchanged when the participants with higher education perceived their health as good as compared to the ones who have only graduated from elementary school, or not even that, who often perceive their health as poor.

In 1999 about 33% of the surveyed participants who are suffering from a chronic disease named the lack of funds as the reason for the lack of abidance to the suggested regime of lifestyle, while in 2015 this percentage was 74%.

When we took into consideration to who the participants go when they were ill, we saw the difference between the two years the study was conducted : in 1999, 68.4% and in 2015 74.3% of them asked help from a doctor in a community health center, a private doctor (4.1% and 11.1%, respectively), while 22.4% and 8.0%, respectively went to the doctor in a specialized consultative service in the public sector of healthcare, and 5.1% and 6.6% respectively, did not ask for medical help (Table 3). This difference was highly statistically significant ($\chi^2 = 22.469$; df = 3; $p < 0.01$).

Table 3

Distribution of answers in in two observed period (1999 and 2015) considering whom do the participants go to when they are ill

Addressing in the case of illness	1999	2015	Total
	n (%)	n (%)	n (%)
A doctor in a community health Center	134 (68.4)	168 (74.3)	302 (71.6)
A doctor specialist in public health care sector	44 (22.4)	18 (8.0)	62 (14.7)
A doctor in private health care sector	8 (4.1)	25 (11.1)	33 (7.8)
No one	10 (5.1)	15 (6.6)	25 (5.9)
Total	196 (100)	226 (100)	422 (100)

$\chi^2 = 22.469$; df = 3; $p < 0.01$.

The distribution of the number of visits to the general practitioner in the public sector of healthcare in the two observed years pinpoints the significant changes in the use of services provided: the number of those who, in the previous 6 months did not visit the general practitioner in the government sector of healthcare increased by 6.1%, and the number of those who visited the general practitioner at least once decreased by 13.2%. Thus, the increase in the average number of visits to the general practitioner, from 2.1 per year in 1999 to 3.7 in 2105 was actually a consequence of more frequent visits by the people who had already used to using the services of general practitioner in the government sector of healthcare. Namely, the number of those who used these services 10 or more times a year increased from 4.6% in 1999 to 13.7% in 2015.

As the main cause of dissatisfaction with health care services provided in the public health sector in 2015, respondents identified lack of money, and after that, health professionals and poor work organization. Compared to 1999, this difference was statistically highly significant ($\chi^2 = 29.572$, df = 3, $p < 0.01$) (Table 4).

Table 4

Distribution of answers in 1999 and in 2015 about the main causes of dissatisfaction with health services in the public health care sector

The main causes of dissatisfaction with health services in the public health care sector	1999 n (%)	2015 n (%)	Total n (%)
Health professionals	23 (25.8)	13 (16.2)	36 (21.3)
Lack of money	37 (44.3)	49 (58.6)	86 (50.9)
Poor organization of work	25 (29.9)	7 (8.1)	32 (18.9)
Other	0 (0)	15 (17.1)	15 (8.9)
Total	85 (100)	84 (100)	169 (100)

$\chi^2 = 29.572$; df = 3; $p < 0.01$.

The average amount of money spent per participant in both public and private health sector in 1999 was 694.8 dinars (€29) and in 2015, it was six times more – 14,448.00 dinars (€180.6). The amount for the public health sector in 1999 was 138.9 dinars (€5,8) and in 2015 it was 2,784.00 dinars (€34.8). In 1999 for the private health sector it was 555.9 dinars (€23.2), and in 2015, it was 11,256.00 dinars (€140.7).

Discussion

The results of this study correlate to results of various different studies which point to the fact that the level of education is in a proportional relationship with the self-assessment of health [13, 14] and that it is possible that the inequality in healthcare is a consequence of the perceived fact that the people with higher education have more skills for solving everyday challenges which could have a negative influence on the health of an individual [15].

Unemployment influences the health in two main ways: by a lack of material funds and ability to meet everyday needs as well as the emotional stress related to a lack of job, uncertain future, loss of self-esteem or identity [16].

Furthermore, the studies point to a direct proportional relation between a provided healthcare and individual demands for healthcare [7] as well as between the use of healthcare services and socioeconomic determinants of health [11].

Other studies show that the ever present decrease in the number of the healthcare users has its origin in: the growing skepticism of the public towards new medication and therapeutic treatments, the increase in the user autonomy and readiness of the public to accept upon itself more responsibility for its own health, the change of relationships between income, education and use of public healthcare, the aging of the population with the accompanying increase in the number of chronic diseases and in the decrease in the sizes of families [11].

It is important to highlight some issues concerning inequalities. This is the idea that one should think of health inequalities as deriving from material conditions of life, not psychological factors [17]. But, there are two significant notes about that: first, health inequalities are not limited to those living in absolute deprivation, and, second, material conditions and psychosocial factors are intimately related [18]. Part of the problem of inequalities in health has to do with education, with conditions at work, with job insecurity and unemployment and the nature of neighborhoods [18].

Good health involves reducing levels of educational failure, reducing insecurity and unemployment and improving housing standards. Societies that enable all citizens to play a full and useful role in the social, economic and cultural life of their society will be healthier than those where people face insecurity, exclusion and deprivation [19]. Stressful circumstances making people feel worried, anxious and unable to cope, are damaging to health and may lead to premature death as well as lack of control over work and home [19]. A shortage of food, excess intake and lack of variety cause malnutrition and deficiency diseases [19]. The other main social determinants of health in our society today are: early life, social exclusion, working condition, unemployment, social support, addiction and transport policy [19].

Nowadays, there are eight topic groups that are recognized for actions in key areas relating to health in Europe [20]: young age, education and the family; employment and working conditions, including occupation, unemployment and migrant workers; disadvantage, social exclusion and vulnerability; gross domestic product (GDP), taxes, income and welfare; sustainability and community; preventing and treating ill health; gender and older people.

This is why effective interventions for overcoming the consequences of inequality in the society are necessary. Policy and actions have to attack the causes of ill-health before they can lead to problems on the following levels: wider society, systems, and life course stages (prenatal, early years, working age and older ages). This is challenging task for both decision-makers and public health actors. The healthcare institutions have a role as partners only in one part of activities. Nowadays, there are very important discussions on how social determinants such as birthplace and income can have a greater effect on our lives than access to health care. These would be conducted on a specific target group on a single area following the next steps: superficial interventions (directed on the "symptoms", neglecting the causes of a certain phenomenon), operative interventions (directed on specific activities), preventive interventions (directed on causes of a certain phenomenon) and basic interventions (no direct effects, but they secure the prerequisites and the framework of all the previous interventions).

Conclusion

Health inequalities arise from the conditions in which people are born, grow, live, work and age, and the social determinants of health –inequities in power, money and resources. This survey showed a direct connection between

self-assessment of one's healthcare and their socioeconomic status (employment, education, income). Particularly worrying are two facts: increasing number of participants who do not turn to any health professionals when feeling ill and the fact that the largest number of public healthcare services used belongs to those participants who use those services often, the ones that "circle" in this system.

Health is essential to well-being and to overcoming other effects of social disadvantage. Equality is a key value in assessment of the impact on health. Including end-users as an active members in the healthcare system is one of the ways to improve the quality of health care services, but only if it is conducted on sustainable and culturally accepted manner.

Concflict of interest

The study was not funded by any organization. The authors declare that there is no conflict of interests.

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The effect of acceleration on color vision

Uticaj ubrzanja na kolorni vid

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Abstract

Background/Aim. Over 80% of all information a pilot receives during the flight is visual with color perception being one of the most important visual functions for managing an aircraft. The reception of color is of high significance in aviation due to the importance of signal tracking on instrument panels as well as the importance of visual stimulus and environment signs. There is no sufficient number of papers and studies that deal with this issue, although recent studies have shown that the connection between acceleration and color perception exists. The aim of this study was to demonstrate the correlation between pilot exposure to +Gz acceleration in human centrifuge and color perception before and after acceleration exposure. **Methods**. Subjects of the study were 40 military pilots, aged 35–45, with 10 and 20 years of flying experience. Pilots were exposed to +Gz acceleration (inertial force acts from head to feet) in the human centrifuge for pilot training with accelerations of +2Gz, +5.5Gz up to +7Gz. The tests focused on color perception before and after the exposure to the acceleration. **Results**. Out of 40 pilots examined for color vision, in 35 (87.50%) had normal results in color identification before and after +Gz; 5.00% (2 subjects) had two mistakes – reading number 5 instead of number 3, which falls within the normal trichomes, and reading number 16 instead of number 26. Three subjects (7.50%) gave their answers slower than the accepted response time. After the +7Gz exposure, 34 (85%) persons had normal results in color identification, 2 (5%) subjects made three mistakes – at numbers 5, 74 and 26; one (2.50%) pilot made four mistakes on numbers 5, 7, 74 and 26; 7.50% (3 pilots) of the subjects identified colors slower. **Conclusion.** Color perception in pilots is unstable on high +Gz accelerations. Exposure to +5.5Gz acceleration does not lead to significant changes in color perception, while exposure to +7Gz acceleration showed a significant percentage of reversible disturbance in color perception which lasted for 10 minutes.

Key words:
pilots; aerospace medicine; space simulation; acceleration; color vision.

Apstrakt

Uvod/Cilj. Preko 80% svih informacija u toku letenja kod pilota je vizuelno, a raspoznavanje boja je jedna od vidnih funkcija koja je veoma značajna za upravljanje avionom. U avijaciji je raspoznavanje boja značajno zbog praćenja signala na instrument tablama kao i obojenih vizuelnih stimulusa i znakova okoline. Za sada ne postoji dovoljan broj radova i istraživanja koja se bave ovim pitanjem, premda novije studije pokazuju da veza između ubrzanja i raspoznavanja boja postoji. Cilj našeg istraživanja bio je da se utvrdi da li postoji veza između izlaganja pilota +Gz ubrzanju (sila inercije deluje od glave do stopala) u humanoj centrifugi i promena u raspoznavanju boja pre i nakon izlaganja ubrzanju. **Metode.** Analizirano je 40 pilota vojnog vazduhoplovstva starosti od 35–45 godina, sa letačkim stažom između 10 i 20 godina. Piloti su izlagani +Gz ubrzanju i to +2Gz, +5.5Gz do +7Gz u humanoj centrifugi koja služi za trenažu pilota. Ispitivano je raspoznavanje boja pre i posle izlaganja ubrzanju. **Rezultati**. Od 40 pilota kod kojih je ispitivan kolorni vid, kod 35 (87,50%) pilota raspoznavanje boja pre i posle izlaganja +Gz ubrzanju bilo je normalno, dva (5,00%) pilota su imala dve greške, jedan je broj 5 čitao kao broj 3, što spada u normalne trihomate, a drugi je broj 16 čitao kao 26, a tri (7,50%) pilota su samo sporije davala odgovore. Nakon izlaganja +7Gz ubrzanju 34 (85,00%) pilota normalno je raspoznavalo boje, dva (5,00%) pilota je napravilo tri greške na brojevima 5, 74 i 26 i jedan (2,50%) pilot je imao četiri greške na brojevima 5, 7, 74 i 26, dok su tri (7,50%) pilota samo sporije raspoznavala boje. **Zaključak.** Raspoznavanje boja nije stabilno na visokim + Gz ubrzanjima. Izlaganje +5.5Gz ubrzanju ne dovodi do značajnih promena u raspoznavanju boja, dok je izlaganje +7Gz ubrzanju pokazalo značajan procenat u poremećaju raspoznavanja boja, koji je bio reverzibilan, jer je nakon 10 min raspoznavanje boja bilo u celosti normalno.

Ključne reči:
piloti; medicina, vazduhoplovna; pilotiranje, simulisano; ubrzanje; vid, kolorni.

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Introduction

Over 80% of all information a pilot receives during a flight is of visual character and color recognition is one of the most important functions for managing an aircraft. Color vision or color recognition is the ability to react to different spectral light regardless of its intensity.

Color recognition in aviation is of extreme importance due to monitoring the signals on the dashboard as well as monitoring the plane's surroundings while in flight. Normal color recognition is explained in trichromatic theory based on the fact that all visible colors can be formed by combination of 3 basic colors – red, green and blue. The development of modern aviation forces pilots into situation where they are exposed to loads that are nine, ten, twelve and more times greater than the gravity force on Earth [1–10]. Testing the color vision is one of the obligatory visual functions tested when selecting candidates for aviation services and is regularly controlled in every medical examination. Long lasting acceleration mostly occurs in air maneuvers of fighting airplanes. Such acceleration leads to effects on internal organs and tissues (effects such as torsion and retrievals), mostly in changes of liquid tissues, causing significant redistribution in the body. Physiologically, blood redistribution and increased blood pressure leading the blood from head to toes are the most important effects that +Gz acceleration has on human body, which lead to the poor perfusion in CNS and eye as its most sensitive part causing disturbances such as grey veil, tunnel sight, black veil. Poor perfusion causes hypoxia that negatively affects the cells of the eye [11].

Bad color perception can be caused by atmospheric conditions such as bright sun with blinding light phenomena, night, cloudy weather and especially misty weather. The fog makes the position signal difficult to see and the colors get reddish hue. From the avio-physiological point of view, forces of inertia are important due to their effect on the human body, and to which organism are exposed during acceleration.

Newer and modern aircrafts use colored displays, some of which are mounted onto the pilot's helmet, making color vision and correct interpretation of data even more important for safety and flight efficiency, especially in military air force and during combat. +Gz acceleration leads to decreased blood float in the brain, therefore causing a reduction of blood in the blood vessels of retina, leading to temporary changes in visual functions such as loss of central and peripheral vision, thus increasing the probability of losing the function of color recognition. We still do not have the possibility to follow the blood flow through the retina during +Gz exposure as well as its influence on color recognition.

This research is based on a survey of pilots in conditions of exposure to +Gz acceleration in human centrifuge used for pilot training for the high performance flying and strong G load aircrafts. The centrifuge is a combination of gravity and altitude laboratory, reaching accelerations up to 20Gz and simulating heights up to 30,000 meters. Training in the centrifuge aims to increase individual's abilities to ignore +Gz acceleration.

The research was done with simple exposure to only +2Gz acceleration and composite intermittent exposure to acceleration from +2Gz to +5Gz on the first day and +7Gz on the second day.

Aim of this research was determining if there was a connection between pilot exposure to +Gz acceleration in human centrifuge and the changes in color identification, measured before and after the exposure. This question was not a subject of a greater number of articles and studies, although newer studies [1] show that the connection between acceleration and color perception exists.

Methods

Subjects for this research were 40 military pilots age 35–45 with flying experience from 10 to 20 years. All the participants gave written consent for inclusion in the research. The pilots were exposed to +Gz accelerations of +2Gz, +5.5Gz and higher, up to acceleration of +7Gz in human centrifuge of the Institute of Aviation Medicine in Belgrade.

Color perception was examined before and after the +Gz exposure. Participants were exposed to the effects of long-term acceleration in the centrifuge and to coriolis effect in the time between 9 am and 11 am, two hours after meal. Model used was the experimental model EM-1, programmed to achieve constant radial acceleration of +2Gz, counterclockwise, and to perform head motion (active coriolis) after 30 seconds. Every head motion was made in one second, and the position was kept for 30 seconds after the change. The movements of the head were downwards (chin touching sternum), neutral head position, head on right shoulder forming 45 degrees angle and movement back to neutral position. After that movement, the device is slowed down to +1Gz, signal lamps for controlling the peripheral vision turn on and the acceleration starts in moderate grade of 0.1 G/sec up to the acceleration of +5.5Gz.

Pilot-subject was given a task to respond to light signals from the console within 0.9 seconds as an indicator of preserved peripheral vision. Console lights lighted up alternately on the dashboard. Afterwards, a warning of the same grade followed up to plateau of +2Gz, where immediately after reaching the acceleration of +2Gz, the before mentioned head movements were made.

On the second day of testing, the pilots were exposed to +5.5Gz acceleration and the effects of +7Gz acceleration while wearing Anti G suit. Pseudochromatic table Ishihara with 39 pages was used for testing color vision. Research was done in accordance with the instructions given for the test and carried out in the laboratory for altitude research.

All pilots tested for this research passed tests of color vision during their selective examination, by method of denomination and equalization. There are several tests for color vision research. Some tests are designed primarily for congenital i.e. inborn and some for acquired color perception deficits.

Tests were chosen based on its possibilities and procedures. Person performing the testing should be patient and have perfect knowledge of the method used, as well as normal color vision. These tests were based on decimation

method. The based principle was usage of small circles different in size, light and tonality. Circles were placed in specific positions to form various signs such as numbers and lines, easily noticed by normal trichomes. Ishihara test is placed 60 to 75 cm from eyes of the participant, and each page in the book is presented in 3 to 5 seconds with artificial light in a noise isolated room. Every subject had to read over 18 numbers to be considered as a person with normal color vision. Reading less than 9 pages would mean that there is a deficit in color perception.

Pilots have read Ishihara tables just before entering the centrifuge, immediately after exposure to +Gz acceleration and 10 minutes after leaving the centrifuge.

Results

Color identification tests showed that, out of 40 pilots tested for color vision, 35 (87.50%) pilots had normal color identification before and after +Gz exposure. Two (5.00%) pilots made 2 mistakes each, where one pilot read number 3 instead of 4 – which falls within normal trichomates, and the other pilot read 26 instead of 16. Three (7.50%) pilots gave slow answers after being exposed to +5,5Gz acceleration. On the second day, when the pilots were exposed to +7Gz acceleration, 34 (85%) out of 40 tested pilots had no changes in color recognition, two (5.00%) subjects made three mistakes reading numbers 5, 74 and 26. One (2.50%) subject made four mistakes while reading numbers 5, 7, 74 and 26. Three (7.50%) pilots gave slow answers.

Exposure to the +5.5Gz acceleration during the first day of testing did not show significant changes in color recognition before and after the exposure to +Gz acceleration. Exposing the subject to +7Gz acceleration caused a greater percent of mistakes and worse color recognition. Totally 85% of the tested pilots had normal color recognition. Even though the tested pilots were experienced and had good previous training, the results show that greater accelerations contribute to defects in color vision. The defects were reversible – ten minutes after the tests were done, color recognition in pilots was back to normal and no mistake was made when reading numbers on the Ishihara tables (Table 1).

Table 1

Number (%) of pilots making errors in color perception

Acceleration	Errors made, n (%)					
	0	1	2	3	4	slow reading
Before exposure to the acceleration +Gz	40 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Immediately after exposure +5.5 Gz	35 (87.50)	2 (5)	0 (0)	0 (0)	0 (0)	3 (7.50)
Immediately after exposure +7 Gz	34 (85)	0 (0)	0 (0)	2 (5)	1 (2.5)	3 (7.50)
10 minutes after exposure to + Gz	40	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Discussion

In our study, when exposed to +5.5Gz acceleration, color vision was normal in 87.50% of cases; 12.50% of cases showed a change either in slower reading of Ishihara tables or in reading the numbers wrong. After the exposure to +7Gz acceleration, 85% of subject showed normal color vision; 7.50% of tested pilots read the numbers slower, and 7.50 of them showed a defect in color perception. Recent studies [2–4] showed that the acceleration has effects on color perception, which mostly occurs at high +G acceleration, particularly on +9Gz, where wrong answers were given in 7.7% of the cases. Retinal blood flow decreases during accelerations that high, but thanks to the good training, it returns to normal level. This ability is very important for military maneuvers where everything happens fast and under additional stress.

Expectations for the results of this research were that the effects of acceleration are mostly noticeable on +7Gz acceleration, due to cessation of blood flow through retinal blood vessels. Further researches are made to test the effects of blood flow and changes in the blood stream of retina on color vision [12].

Differences in the results of this and other researches can be explained as the consequence of using different methodologies. This research tested color vision using a method of discrimination with pseudoisochromatic Ishihara tables and the pilots were exposed to acceleration in human centrifuge which is used at the Institute of Aviation Medicine.

Persons with color perception deficiency either do not perceive the numbers or see completely different numbers which is typical for the anomaly. These different perceptions occur due to apparent pseudoisochromasia (false color equality). Ishihara tables are constructed in such a way to easily detect red and green color defects, and major disadvantage of this test is that it is not quantitative and cannot separate anomals from anops with certainty, cannot distinguish protanops (lack of noticing red colors) from deuteranops (lack of noticing green colors) and cannot separate people with defect in blue (trinatops) and yellow color from people with normal color recognition. All of the participants were tested with color recognition and denomination method during the first selective tests. Denomination method is used for determining whether the subject recognizes basic colors on Baynes lantern method of exaltation on Oculus HMC anomaloscope, because of its quantitative as well as qualitative analysis of eventual deficits in color perception. Method of denomination is indicated in certain professions and in this case the pilot profession serves to verify the sense of color in special conditions and circumstances. These are mostly persons working with signal utilities in a variety of light and weather conditions (scotopic, mesotopic, photopic conditions, fog, rain, etc.) Testing is done with special utili-

ties (lamps, lanterns) for better imitation of the before mentioned natural conditions. This test is reliable for extracting trichomates from persons with severe defect in color perception, but is not reliable for diagnosing the type of disorder and estimating its severity. The equalization test is also applied with selective examination which uses spectral colors and provides quantitative as well as qualitative analyses of eventual deficits in color vision. At the Institute of Aviation Medicine in Belgrade where one testing was done, Oculus HMC anomaloscope was used and the diagnosis with severity of the deficit was tested by determining AQ (anomaly coefficient) in subjects. All pilots exposed to +Gz acceleration in human centrifuge were determined AQ which was within normal limits (from 1,00 to 1,31). This research started with the assumption that all pilots have normal color perceptions and do not have a quantitative lack of color recognition. It is necessary to emphasize that color perception declines with decreasing brightness; first comes the loss of ability to distinguish red color, then green and at last, blue. The effect of reducing the lighting was not examined due to the tests being done under the same lighting conditions. High +Gz stress has two primary effects. The instantaneous effect is pressure drop at head level and it is proportional to G load; second primary, but somewhat postponed effect, is withdrawal of blood in legs and abdomen. Acute redistribution leads to a decrease in the inflow of venous blood to heart, reduced cardiac output and reduction of the blood pressure at the level of the heart that can further lead to blood pressure reduction in the head level. Physiologically, the ultimate effect on the level of visual function is loss of peripheral and central vision and loss of consciousness. It should be pointed out that energy reserves of the eyes and brain get exhausted in about 5 seconds since the start of lack of blood in the head of the pilot exposed to +Gz acceleration, and in that time the baroreciptoral reflex has not yet activated. In this case, the initial visual symptoms can be absent and could cause a current loss of consciousness which is the most dangerous for pilots. According to some authors [1, 5], in the conditions of greater +Gz acceleration, pilots see red colors as orange or yellow. Studies that dealt with comparative lighting relation, contrasts and color shades noted that changes in perceptions of greater +Gz acceleration, such as +9Gz, the pilots couldn't distinguish the color blue on the display, for they have seen it as white, and the color yellow as green. The ability to recognize colors decreases with decreasing lighting, first the ability to identify red color fails with light reduction, then the color green and at the lowest light, the color blue. Violet color has the longest photochromatical effect, i.e. it is also the longest seen color in low intensity lighting, therefore the modern airports use violet lamps for marking the runway for takeoffs and landings [13–20].

To improve the tolerance on +Gz in human centrifuge used for training and exercise of pilots, it is necessary to emphasize that lighting and contrast sensitivity in cabin are important for color perception, as described in some studies [6].

Conclusion

Color identification is not stable at high +Gz accelerations. Before any definitive conclusions on the perception of color can be reached, further researches should be made, testing the cockpit of the airplane where central and peripheral vision may have an impact. Testing on the Oculus HMC anomaloscope, that is, using the equalization method after leaving the centrifuge and immediately after the +Gz acceleration exposure could be applied in some of the researches to follow. At positive +Gz accelerations the force of inertia acts parallel to longitudinal axis of the body, in such a way that the pilot is pinched in the seat. Due to the great practical significance, especially in air battle, this acceleration attracts great attention and its effect on visual functions is constantly tested. Training in human centrifuge is also of great importance, because with increasing the individual exercise, the pilots are trained to tolerate high +Gz accelerations, which is of great importance in combat maneuvers and every form of training reduces the possibility of color blindness due primarily to loss of retinal blood stream for visual symptoms of tunnel sight, grey and black veil, and finally loss of consciousness.

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CASE REPORT

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Bochdalek hernia in adults – A case report

Bochdalek-ova hernija kod odraslih

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Abstract

Introduction. Asymptomatic Bochdalek hernia in adults is a rarity. The aim of this paper is to present a rare case of Bochdalek hernia among adults and to point out to significance of clinical suspicion and important role of imaging techniques in reaching the exact diagnosis of this abnormality. **Case report.** A patient, aged 68 years, came to the Clinic of Pulmonology complaining of constant dyspnea, coughing and fatigue. Computed tomography (CT) findings were dominated by the large rear right diaphragmatic hernia with the hernial sac that reached the carina trachea and urged the principal bronchi. The stomach, duodenum and proximal part of jejunal winds, as well as a greater amount of omental and mesenteric adipose tissue were localized in hernial sac. Radiography of gastroduodenum showed: entry of the esophagus into the cardia was in the level of the right main bronchus. Stomach was mainly located in the chest (cardia, upper corpus half - to the level of the right main bronchus). Operation was indicated. First, we made right thoracotomy and the hernia sac was separated from the right lung and then we performed median laparotomy and the contents of the hernia sac were returned to the abdominal cavity; diaphragm defect was reconstructed with prolen mesh. **Conclusion.** We presented a rare case of right sided Bochdalek hernia which was discovered at late age and surgically treated with success.

Key words:
hernias, diaphragmatic, congenital; diagnosis; thoracic surgical procedures; digestive system surgical procedures; adults; treatment outocme.

Apstrakt

Uvod. Asimptomatska Bochdalek-ova hernija kod odraslih je raritet. Cilj rada je da se prikaže redak slučaj Bochdalekove hernije kod odraslih i da se naglasi značaj kliničke sumnje i značajna uloga imidžing tehnika u postavljanju tačne dijagnoze ove abnormalnosti. **Prikaz bolesnika.** Bolesnica, starosti 68 godina, se javila u Kliniku za pulmologiju zbog gušenja, kašlja i malaksalosti. U nalazu kompjuterizovane tomografije (CT) dominirala je velika zadnja desna dijafragmalna hernija čija kilna vreća je dopirala do nivoa karine i razmicala principalne bronhe. U kilnoj vreći se nalazio želudac, duodenum i deo proksimalnih jejunalnih vijuga, kao i veća količina omentalnog i mezenterijalnog masnog tkiva. Radiografijom gastroduodenuma ustanovljen je ulazak jednjaka u kardiju u nivou desnog principalnog bronha. Želudac se većim delom nalazio u grudnom košu (kardija, gornja polovina korpusa do niova desnog principalnog bronha). Indikovana je operacija. Urađena je prvo desna torakotomija i kilna kesa je odvojena od desnog plućnog krila, a zatim je urađena medijalna laparotomija i sadržaj kilne kese je vraćen u trbušnu duplju. Defekt na dijafragmi je zbrinut prolenskom mrežicom. **Zaključak.** Predstavljen je redak slučaj desnostrane Bochdalek-ove hernije, otkriven u kasnijoj životnoj dobi i hiruški tretiran sa uspehom.

Ključne reči:
hernije, dijafragmalne, kongenitalne; dijagnoza; hirurgija, torakalna, procedure; hirurgija digestivnog sistema, procedure; odrasle osobe; lečenje, ishod.

Introduction

Congenital diaphragmatic hernia is frequently mentioned in medical literature since its first description dating back in the early 18th century [1]. It includes a number of birth defects, the main characteristics of which are abnormalities in the development of the diaphragm [2]. It occurs due to the impossibility of closing of pleuroperitoneal fold between 4th and 10th week of gestation. It is the most common intrathoracic, extracardiac anomaly associated with high risk of

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morbidity and mortality [3]. Congenital diaphragmatic hernia makes 8% of all major congenital anomalies. It generally occurs in the first hours after birth, whereas traumatic hernia can occur at any age. Subsequent clinical presentation of congenital diaphragmatic hernia is very rare [2]. In adults, there are various causes such as: penetrating or blunt injury, physical effort, coughing episodes, sneezing, pregnancy and even eating too much food [4]. In 85% of cases congenital diaphragmatic hernia is localized on the left. The anatomical position of the liver and the barriers it creates are the reasons why congenital diaphragmatic hernia is localized on the right side in only 15% of cases [2]. The contents of the hernial sac differ depending on which side the hernia is localized [5]. Bilateral hernias rarely occur and usually have fatal consequences. The most common form of congenital diaphragmatic hernia is a posterolateral or Bochdalek hernia. In most cases, Bochdalek hernia occurs in the neonatal period with poor prognosis [1]. Asymptomatic Bochdalek hernia in adults is a rarity. Common clinical manifestations are problems with breathing or pneumonia. Symptoms of the gastrointestinal tract may be also present. Although rare, Bochdalek hernia may need to be considered in the differential diagnosis for patients in the old age who have foregut symptoms. A careful and unbiased interpretation of radiologic tests is essential to recognize the disease and perform the correct operation [6]. Imaging plays an important role in diagnosing and assessing the contents of the hernia and at the same time evaluating the presence of any associated abnormality [7]. The final diagnosis is set by computed tomography (CT) [2].

The aim of this report is to present a rare case of 68-years old patient with presentation of right-sided Bochdalek hernia, which is a rarity in clinical practice, as well as to point out to significance of clinical suspicion and important role of imaging techniques in reaching the exact diagnosis of this abnormality.

Case report

We presented a patient aged 68 years who came to the Clinic of Pulmonology at the Clinical Centre Kragujevac complaining because of constant dyspnea, coughing and fatigue. These symptoms lasted for several years, with intermittent periods without symptoms. Dry irritating cough and intensified malaise appeared ten days before admission. After completion of chest X-ray and having observed infiltrative paracardial lesion on the right side, the patient was hospitalized for further diagnostic and therapeutic procedures.

During the interview, the patient stated that during first years of her life she had often respiratory difficulties such as dyspnea and cough, mostly during night, with frequent respiratory tract infections due to which she had to seek medical help. She had no medical documentation on diagnosis or treatment. Since puberty, these difficulties were significantly less frequent and milder in intensity. They intensified again during last 5 years. She was treated for hypertension over last 10 years and over last 3 years for diabetes. There was no information on significant injuries neither on previous chest X-ray findings.

Auscultatory findings of the lungs were: diffusely impaired breathing sound, with occasional wheezing. Auscultation of the heart showed: rhythmical action, clear tones, systolic murmur at the top of the heart and over aorta. There were no other significant physical findings.

Radiography of the heart and lungs revealed: intense homogeneous soft-tissue infiltrative opacity over basal area of the right lung which closed right costophrenic sinus while left costophrenic sinus was free and the size of cardiac shadow was within the normal range (Figure 1). Bronchoscopic findings showed stenosis of bronchus for right lower lobe due to extramural compression. The rest of the findings were normal. Ultrasound findings of the abdomen were: liver, pancreas and spleen had normal echo structure. Gall bladder wall was thickend with two calculi of larger diameter - up to 2.8 cm. CT of the chest showed absence of pathological changes in the lungs, trachea and bronchi. There was no pleural effusion. CT findings were dominated by the large rear right diaphragmatic hernia with hernial sac that reached the carina trachea and urged the principal bronchi. The stomach, duodenum and proximal part of jejunal winds, as well as greater amount of omental and mesenteric adipose tissue was localized in hernial sac. Conclusion was: Hernia diaphragmalis Bochdaleck l. dex (Figure 2). Radiography of gastroduodenum showed that: entry of the esophagus into the cardia was at the level of the right main bronchus. Stomach was mainly located in the chest (cardia, upper half of the corpus – to the level of right main bronchus). Stomach preserved form with no apparent defect in the filling of blood vessels and no elements of acute ulcer. Stomach was predominantly located in the retrocardiac space. Duodenal bulb, with no visible signs of acute ulcer disease, was located above the hemidiaphragmal conture in the chest cavity. The findings resembled Bochdalek hiatus hernia the most (Figure 3). The patient was treated surgically. Considering preoperative diagnosis of congenital hiatus hernia, discovered at the age of 68, massive adhesions between the hernial sac and thoracic organs were expected.

Fig. 1 – A posteroanterior chest radiograph shows an abnormal soft tissue opacity obscuring the right cardiac margin.

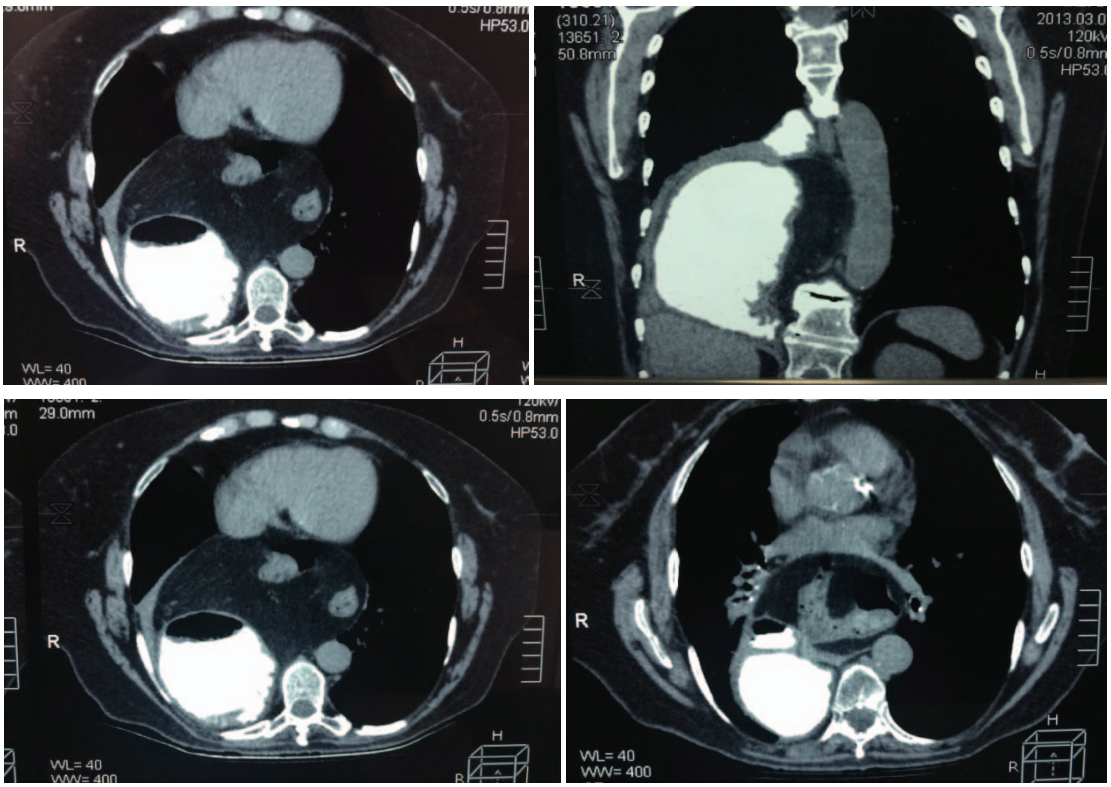

Fig. 2 – Computed tomography (CT) findings are dominated by the large rear right diaphragmatic hernia with the hernia sac that reaches the carina trachea and urges the principal bronchi. The stomach, duodenum and proximal part of jejunal winds, as well as greater amount of omental and mesenteric adipose tissue are localized in hernial sac.

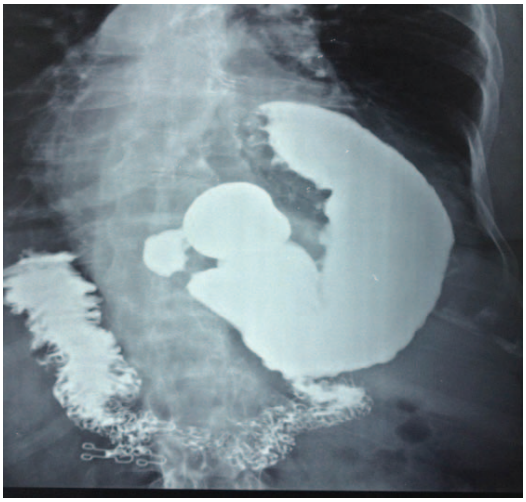

Fig. 3 – Swallowing barium shows on the right side Bochdalek hernia with the major part of the stomach located in the chest.

At first, right side posterolateral thoracotomy was performed through fifth intercostal space. During the operation, exploration showed the right lung with signs of compressive athelectasis with the presence of large diaphragmal hernia localized in posterolateral region. Careful removal of massive adhesions between the right lung and hernial sac was done and then the hernial sac was repositioned into abdominal cavity, after which a defect on diaphragm with dimensions approximately 9×6 cm was noted. Subsequently, medial laparathomy was performed and digestive organs which were involved in the described hernia (stomach with duodenum and part of grater omentum as well as proximal part of jejunum) were separated through carefully adhesiolysis, partially blunt, partially sharply, but without any organ injury, since they were largely conjoined. Defect on diaphragm was reconstructed with prolen mesh. Further exploration of the abdominal cavity showed calculi within the gall bladder, due to which retrograde cholecystectomy was done. There were no postoperative complications. The patient was discharged without any respiratory or gastrointestinal difficulties. Chest X-ray examination 15 days after the surgery showed no signs of herniation of abdominal organs into chest cavity.

Discussion

Congenital diaphragmatic hernias, clinically represented in adult life, are extremely rare. They can occur through the frontal parasternal opening (Morgagni) or through the posterolateral opening (Bochdalek). Posterolateral, Bochdalek hernia is the most common type of congenital diaphragmatic hernias (95%) [1]. Most Bochdalek hernias are present during neonatal life, have poor prognosis and are often associated with congenital anomalies of the lungs. Overall prevalence of Bochdalek hernias in adults is 6% [2]. Clinical manifestations of Bochdalek hernias in children and

adults are different. In children it is usually presented by breathing disorders and cyanosis [7], while in adults it can be presented in 2 ways. Patients frequently have vague, mostly gastrointestinal symptoms. The most common gastrointestinal symptoms in adults are: recurrent abdominal pain, flatulence after a meal and vomiting [8]. Chest pain, dyspnea and wheezing, which are the symptoms of the respiratory tract, may occur in adults as well, which was the case with our patient. These symptoms may be followed by attacks or episodes of incarceration, with serious consequences.

These symptoms may be temporary. It is believed that late presentation of symptoms or the absence of symptoms can occur due to occlusion of diaphragmal defect within the abdomen.

The diagnosis of Bochdalek hernia can be made by conventional radiological methods like plain radiographs and barium studies [7].

Congenital diaphragmatic hernias are uncommon diagnosis among the adults, mostly because they occur in childhood. Unclear and nonspecific clinical presentation results in late diagnosis. They are diagnosed accidentally in adults, as an incidental medical finding or when the symptoms appear. The diagnosis of Bochdalek hernia in adulthood is problematic because of the rarity of this disease and the variety of the presenting symptoms. The presence of bowel sounds within the chest and the absence of breath sounds are typical findings associated with Bochdalek hernia [9]. They can be easily detected by radiography. Ultrasound has an important role in prenatal diagnosis of this condition. Regarding postnatal evaluation of diaphragmatic hernia, ultrasound helps in marking of the whole diaphragm, allowing an estimation of which organs are causing a herniation inside the thoracic cavity. Computed tomography is the most accurate way of diagnosing. In adults in whom the diagnosis is missed, CT has a very important role. Without CT, nearly 38% of adults had a wrong diagnosis – pleural effusion, pleural empyema, pulmonary cyst and pneumothorax [7]. Clinical presentation of a right-sided Bochdalek hernia can also manifest as strangulation of the contents of the hernia, colon necrosis and hemothorax. Previous data indicate that the final diagnosis is confirmed by CT [8].

The treatment of Bochdalek hernia is surgical, usually without recidivism and patients are usually without symptoms after the intervention [7]. Traditionally, the surgical management of diaphragmatic defects were performed via laparotomy and/or thoracotomy. Several authors suggested an abdominal approach for left-sided defects and a thoracic approach for right-sided hernias. The improved ability of separating adhesions between the hernial sac and pleura is the main advantage of the transthoracic repair.

In our patient, thoracotomy and adhesiolysis were performed at first, then, the laparathomy with adhesiolysis of largely conjoined organs within the present hernia and closure of diaphragmal defect with polypropylene netting. Regardless to surgical procedure applied, there are controversies on repair of Bochdalek hernia, such as dissection of the hernial sac and the usage of grafts. Regarding the fact that dissection of hernial sac is largely attributed to a high risk of pleural injuries, most surgeons leave hernial sac at its primary position. Smaller defects are easier to repair while the repair of larger ones lead to reduction of volume of intra-abdominal cavity. The choice of procedure depends on the surgeon's experience [9].

Conclusion

We presented a rare case of right-sided Bochdalek hernia which was discovered at late age and surgically treated with success. Even though it is rare, this disorder should be recognised, examined and treated appropriately to avoid complications.

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LETTER TO THE EDITOR
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Mid-regional pro-adrenomedulin as a marker of perioperative mortality in non-cardiac surgery

Mid-regionalni pro-adrenomedulin kao marker perioperativnog mortaliteta u nekardijalnoj hirurgiji

To the Editor:

Recently published study revealed that out of a total number of patients undergoing a major non-cardiac surgery, 5% of them could have myocardial infarction [1]. After 30 days from the major non-cardiac surgery, over 11% of patients would probably die, most likely due to cardiovascular (CV) causes.

There are several widely used methods for estimating preoperative cardiac risk based on risk scores and risk stratification scales, e.g., the Revised Cardiac Risk Index (RCRI) [2,3].

It is known that patients suffering from different diseases (not only cardiovascular ones) with increased mid-regional fragment of the pro-adrenomedullin molecule (MR-proADM) levels (different mechanisms of secretion involved) are at high risk of mortality [4–6]. In a large number of trials, the predictive role of MR-proADM as a biomarker for clinical outcome was investigated in non-surgical patients [7,8]. Results of the GISSI and LAMP studies, conducted in patients with chronic heart failure and myocardial infarction, demonstrated that MR-proADM concentrations higher than 0.75 nmol/L and 0.73 nmol/L, respectively, were predictors of poor outcome [5,9].

Because of that, we performed a prospective observational study with an aim to evaluate MR-proADM as a risk marker for CV mortality in non-cardiac surgical patients older than 55 years who had at least one CV risk factor, which is usually the case in the real-life. MR-proADM was determined before surgery by using a sandwich immunoluminometric assay (MR-proADM, BRAHMS AG, Hennigsdorf/Berlin, Germany). Mean MR-proADM in 264 healthy individuals in previous investigations was 0.33 ± 0.07 nmol/L (range 0.10–0.64 nmol/L) and the assay had a measuring range from 0 to 100 nmol/L [4].

We enrolled 81 patients undergoing major abdominal (n = 56), thoracic (n = 4), orthopedic (n = 20) and vascular (n = 1) surgery under general anesthesia with at least one of the following CV risk factors: diabetes mellitus, hypertension, hyperlipidemia, active smoking, or a family history of cardiac disease. Exclusion criteria were emergent surgery and the inability to understand or sign the informed consent. We routinely calculated the Revised Cardiac Risk Index (Lee score) [3]. The clinical endpoint of the study was mortality within 30 days after the surgery.

The study included 42 women (51.9%) and 39 men (48.1%), aged 71.29 ± 6.62 years (range: 55–87 years). Within 30 days after the surgery 14 (17.3%) patients died, all due to cardiac causes and all being subjected to the abdominal surgery. This high and early postoperative mortality rate in our study could be explained by the fact that study included relatively small number of elderly patients with different co-morbidities who underwent an extensive surgery carrying a particularly high risk for poor outcome. Patients who died were older than 65 years (75.7 ± 6.7) and all of them underwent the abdominal resection (mostly radical) due to malignant abdominal tumors. Our results are in line with those obtained in other studies with elderly patients. Heriot at al. [10] reported that postoperative mortality in elderly patients with colorectal cancer was as high as 15.6%. A mortality rate of 16% was found in patients over 70 years of age undergoing major elective orthopedic surgery [11].

The concentration of MR-proADM was statistically significantly higher in the deceased patients when comparing to survivors ($p < 0.001$). On the other hand, the values of Lee were not significantly different with respect to fatal outcome ($p = 0.283$).

The patients with MR-proADM in the upper quartile had statistically significant shorter survival, comparing to other patients ($p = 0.007$). The survival time of patients with MR-proADM in the upper quartile was 15 (95% CI 6.46–23.54) days and in other patients it was 28 (95% CI 20.7–28.66) days. The group of deceased patients included 9 (45%) patients with MR-proADM above 0.86 (the upper quartile) and 5 (8.2%) with MR-proADM below 0.86. Our patients with higher NYHA class and Lee score above 6.60 had MR-proADM in the upper quartile. They also had the worst 30-day prognosis. In a number of studies on patients with heart failure, good correlation between NYHA class, natriuretic peptides and MR-proADM was shown [12].

In our study, MR-proADM compared to the Lee score was a better predictor of postoperative mortality in the patients (with at least one CV risk factor) subjected to extensive non-cardiac surgery. Therefore, measuring MR-proADM concentrations might help in identifying a high-risk patients before performing non-cardiac surgery. These patients could have benefit from a risk reduction measurements.

We need more studies regarding the prognostic role of MR-proADM in perioperative risk stratification keeping in mind that our study had several limitations (small number of patients, elderly population with different comorbidities who underwent an extensive non-cardiac surgery). Nevertheless, we belive that MR-proADM is a promising prognostic biomarker for the preoperative risk assessment, either alone or together with other risk factors. This biomarker is involved in many (patho)physiological processes and might be not only a marker of CV risk, but general perioperative risk.

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Mlađan Golubović*, Dragana Stanojević[†], Nenad Jovanović*, Milan Lazarević[‡], Velimir Perić[§], Dragan Milić[‡§], Dragana Unić Stojanović[‖¶], Dejan Marković[¶], Biljana Stošić*[§], Nemanja Stepanović[††], Radmilo Janković*[§]**

Clinical Center Niš, * Center for Anesthesiology and Reanimatology, [†]Clinic for Cardiovascular Diseases, [‡]Clinic for Cardiosurgery, [††]Clinic for Vascular Surgery, Niš, Serbia; University of Niš, [§]Faculty of Medicine, Niš, Serbia; [‖]Institute for Cardiovascular Disease Dedinje, Belgrade, Serbia; University of Belgrade, [¶]Faculty of Medicine, Belgrade, Serbia; Clinical Center Serbia, **Department for Anesthesiology and Reanimatology, Belgrade, Serbia

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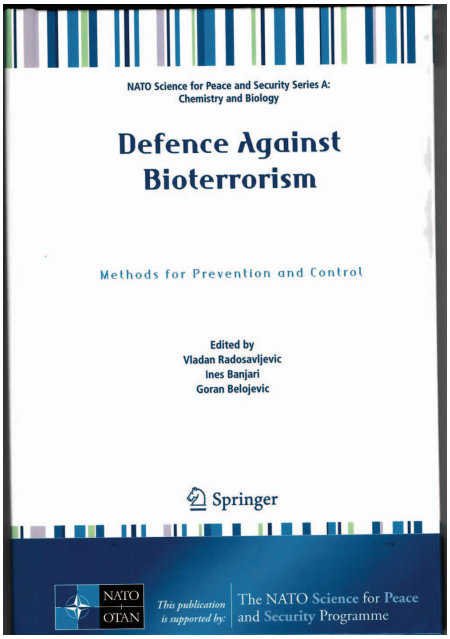
BOOK REVIEW/PRIKAZ KNJIGE

Defence Against Bioterrorism, Methods for Prevention and Control



Title: Defence Against Bioterrorism, Methods for Prevention and Control
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"Defence Against Bioterrorism, Methods for Prevention and Control" edited by Vladan Radosavljevic, Ines Banjari and Goran Belojevic is an up-to-date distinguished international scientific monograph of highest interest for public health professionals, military experts and wider community. This valuable book unites under the umbrella of a respectable publisher Springer three editors and 33 authors from nine countries (Serbia, Croatia, Germany, United States of America, Israel, France, Italy, Bulgaria, Poland) employed by the institutions which would have a crucial role in the case of a threat for international and national security, such as North Atlantic Treaty Organisation - NATO, Brussels, Belgium; Center for Disease Control and Prevention – CDC, Atlanta, Stony Brook University, Stony Brook, New York, and Virginia Polytechnic Institute and State University (Virginia Tech), Arlington, Virginia, United States of America; German Military Institute of Microbiology (BwIM), Munich, Germany; Military Centre for Epidemiology and Public Health, Marseille, and French Surgeon General Office, Paris, France; School of Public Health, University of Haifa, Haifa, Israel; Laboratory of Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli-Sacco, "L. Sacco" – University Hospital, Milan, and Department for Sustainability, Italian National Agency for New Technologies, Energy and the Environment (ENEA), 'Casaccia' Research Centre, Rome, Italy; The Analysis Team, The Epidemiological Response Center of the Polish Armed Forces, Warsaw, Poland; Faculty of Food Technology Josip Juraj Strossmayer University of Osijek, Osijek, and Teaching Institute of Public Health "Dr. Andrija Štampar", Zagreb, Croatia; Military; Military Medical Headquarter and Military Medical Academy, University of Defence in Belgrade, Ministry of Defence of the Republic of Serbia, Institute of Hygiene and Medical Ecology at the Faculty of Medicine University of Belgrade and Strategic Research Institute, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia.

Countering bioterrorism/biothreats is one of the priorities of national health and security systems worldwide. As a contribution to this endeavour, the NATO Science for Peace and Security Programme assigned the editors of this volume the topic "Defence Against Bioterrorism: Methods for Prevention and Control". The top experts worldwide gathered for the NATO Advanced Research Workshop held in Belgrade from 16 to 17 March 2017 and agreed on a creative task of making a multidisciplinary platform (bioshield) against bioterrorism/biothreats and accidental and natural outbreaks. It includes means for differentiation between intentional and natural epidemics, all four levels of prevention, aspects of intelligence and security, preservation of food supply chain, management of panic and ethical aspects. Knowledge and pro-

fessional experience from preventive and clinical medicine, security, intelligence, safety and other areas are systematically classified in primordial, primary, secondary and tertiary levels of prevention. Special emphasis is put on improving the bioshield. Applications of approaches presented in the monograph may reduce the possibility of occurrence and consequences of bioterrorism and accidental and natural outbreaks.

The bioshield presented in the monograph is highly effective (comprised of four levels of prevention, with additional strategies against bioterrorism), affordable (based on the existing public health and security infrastructure), applicable (may be activated within few hours of an outbreak or a bioterroristic attack) and practical (especially important in decision making). Content of this monograph will serve as a tool for development, improvement and/or implementation of the bioshield against bioterrorism/biothreats.

Crucial improvements are made by the reviewers:

- Dr. John McConnell Editor-in-Chief of *The Lancet Infectious Diseases;*
- Dr. Gigi Kwik Gronvall, Senior Associate at the Johns Hopkins Center for Health, Security and visiting faculty at the Johns Hopkins Bloomberg School of Public Health;
- Dr. Seth Carus, Distinguished Research Fellow at the National Defense University, Center for the Study of Weapons of Mass Destruction, Washington, DC.

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3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhođane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

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

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

Literatura

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

Primeri referenci:

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

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

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

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

Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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