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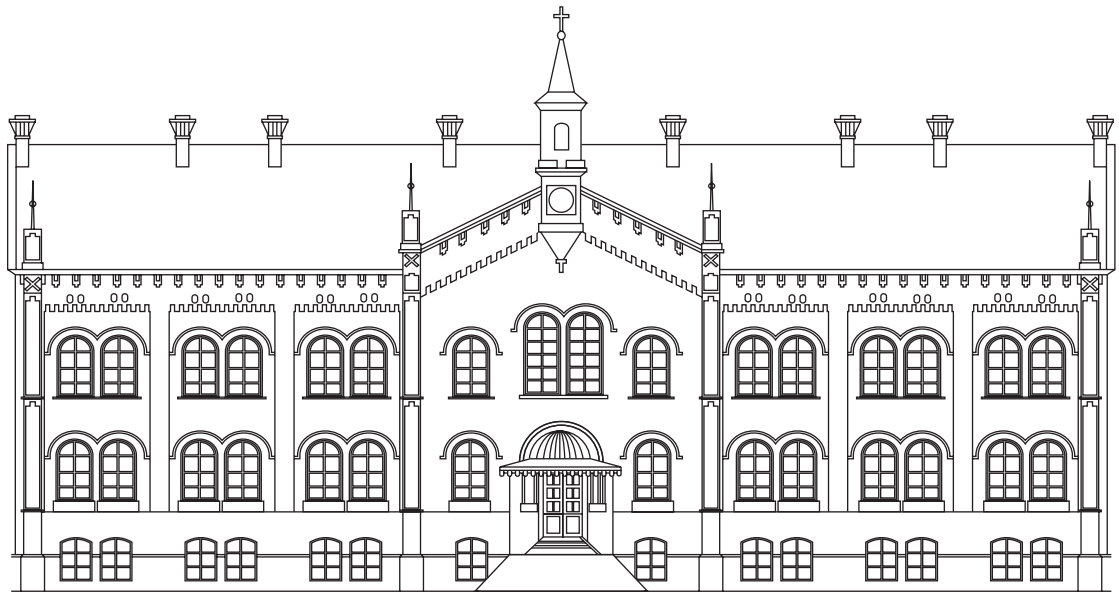

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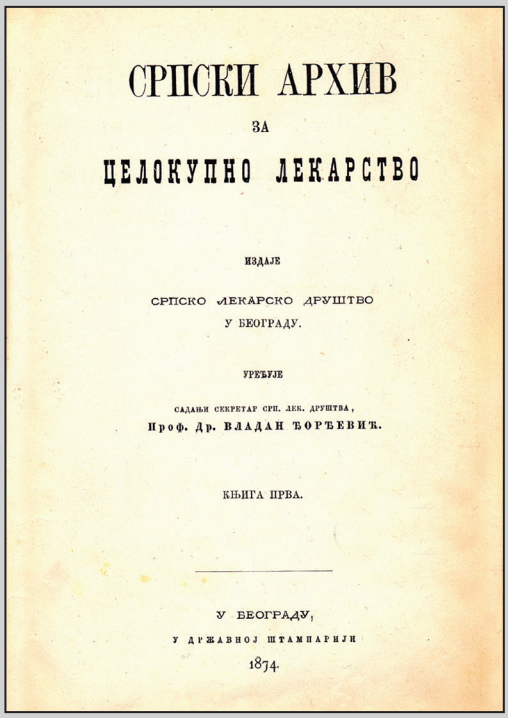

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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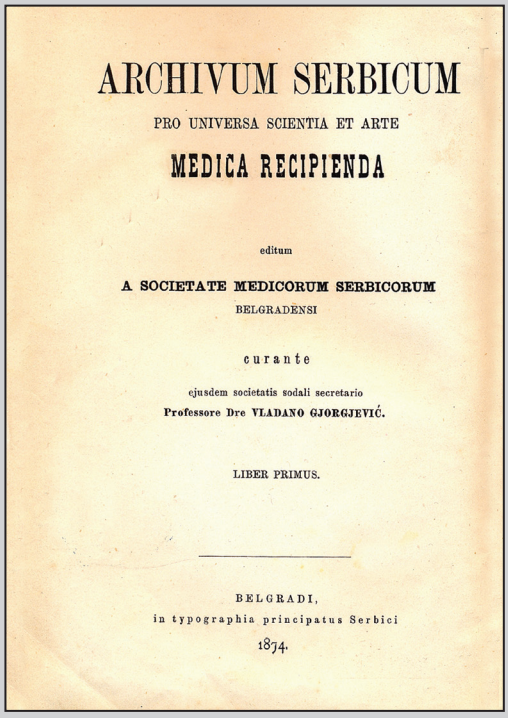
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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BELGRADI,

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1874.

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analyzing strain in samples with all-ceramic systems using the digital image correlation technique

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SUMMARY

Introduction/Objective The study was conducted to identify the maximum strain generated in the samples composed of poly-methyl-methacrylate, Straumann® implants, and three types of ceramic systems.

Methods Three types of experimental models were used, loaded by external load of 100 N, 300 N, and 500 N and analyzed using the digital image correlation method. The models were composed of yttria-stabilized zirconia, e.max lithium disilicate, and Vita Enamic® hybrid ceramics, placed on the Straumann® cylindrical dental implant systems (4 × 10 mm) with straight abutments.

Results Significant differences in strain values between samples with different crown material groups were detected ($p = 0.000$). This suggests that strain values were dependent on the type of crown material. Strain values were also affected by the region of interest ($p = 0.000$). Application of two-way ANOVA enabled testing of the interaction effect between two independent variables, crown material and region of interest, where a significant difference was also found ($p = 0.046$). This indicates that strain values were also influenced by different combinations of material type and region of interest. The highest strain values were found for Z (0.383 ± 0.015) in the apical region, and the lowest for E (0.303 ± 0.015) in the middle region.

Conclusion The study shows maximum strain in the apical and marginal directions. When considered various all-ceramics, we noticed the minimum strain below Vita Enamics®, while the maximum strain was found in samples with yttria-stabilized zirconia crown.

Keywords: all-ceramics; strain; PMMA

INTRODUCTION

The lower fracture toughness in all ceramic systems (full ceramics, metal-free ceramics) can cause crown material breakdown. Hence, it's necessary to create restorative material that could resist possible excessive masticatory forces and satisfy mechanical features due to the irregular shape and size of teeth and dental arches in the restored patients [1]. Still, there is a concern about an impact of currently developed high strength ceramics and their possible influence on underlaying structures, especially considering implant-supported restorations [2]. Thus, additional requirement regarding their biomechanics is to achieve positive effect of all ceramic crowns on the supportive bone tissue that surround teeth or implants. It would be suitable if these materials could be prepared as a mixture composed of restorative dental materials to express their best biomechanical features in a dynamic system of the oral cavity [3]. Furthermore, it is known that the composition of supporting structures may influence stress distribution in all ceramics [4]. Additionally, material properties of all ceramics can cause different strain responses in adjacent structures. The mechanical properties, such as elastic modulus and Poisson's coefficient of each material, should be especially considered in regard to the strain in the supporting tissue [5]. The crown material with lower modulus of elasticity absorbs an increased portion of energy from the applied occlusal load, and transfers less energy to the supporting dental tissue. Therefore, crowns made of acrylic resin/composite showed higher ability to absorb the occlusal stress than crowns made of ceramic material, zirconia, or gold alloy [6]. Considering implant-supported restorations, occlusal materials with high elasticity, like acrylic resin/composite, will mitigate the external occlusal forces and decrease its effect on the bone–implant interface during the occlusal loading conditions [7]. Higher elasticity material reduced the transmitted forces to bone by about 94% compared to zirconia, which improved biocompatibility regarding impacts to adjacent supporting structures [8]. Previous studies investigated the influence of various occlusal materials on stress transferred to implant-supported restorations and supporting structures and found that the type of the restorative material used in implant crown design was a significant factor in the amount and distribution of the stress-loaded structures [9, 10]. The following study was conducted to investigate

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the impact of three usually applied metal-free ceramics on the supporting structure of poly-methyl-methacrylate (PMMA). PMMA was used to substitute the bone due to similar physical characteristics, as previously mentioned [11]. A new classification based on the phase present in the composition of all ceramics included current materials and thus tend to be more suitable for mechanical properties [12]. In accordance with this, all ceramics are divided into three families: glass-matrix ceramics, polycrystalline ceramics, and resin-matrix ceramics. The objective of this study was to determine, evaluate and visualize surface strain generated in samples-models composed of the above mentioned all-ceramics subjected (exposed) to vertical loading conditions. A standardized model for biomechanical investigations was previously proposed [13, 14]. Through the use of the digital image correlation (DIC) method, the authors sought to explain the effect of different all-ceramic crown materials on strain change in peri-implant structures and to indicate which kind of an all-ceramic crown is more suitable for the implant-supported crown. Three sets of null hypotheses were established prior to ANOVA analysis:

1. mean strain values are the same for all samples;
2. mean strain values are the same for all regions of interest;
3. there is no interaction in effect between the ceramic material and the region of interest.

METHODS

The study proposed three groups of experimental models (samples) composed of PMMA, Straumann® implants with three types of all-ceramic posterior crowns (specimens) placed on the Straumann® S Ø 4.1 × 10 mm RN dental implants (Straumann® Cylindrical Dental Implant systems, Basel, Switzerland) with straight abutments. Straumann® RN synOcta® abutments were screwed on a Straumann® dental implant, and tightened using Straumann® SCS screwdriver, ratchet, and torque control device. Abutments were torqued down with 35 Ncm.

All ceramic fully anatomical, contoured crowns were prepared by utilizing computer-aided-design / computer-aided-manufacturing (CAD/CAM) to standardize specimens. The milling was done by a Wieland dental CNC (Ivoclar Vivadent Group, Schaan, Liechtenstein) in a milling unit of a technical dental laboratory. The CAD/CAM milling machine finished ceramic blocks and manufactured all ceramic crowns. The ceramic blocks were processed one by one in the following manner: a block was rotated on its axis while a diamond disk rotated moving up and down around the ceramic block, thus processing it. The movement of the diamond disc was enabled via an electric rail. The precision of milling was in the range of +/- 25 microns. The crowns were polished using polishing sets with a special bur kit for tested all-ceramics, with water cooling. All-ceramic crowns were shaped by milling of the ceramic blocks affixed to a wheel.

The obtained crowns were then placed on abutments using cement and definitively cemented with a special esthetic cement for metal-free ceramics – a self-adhesive Maxcem Elite (Kerr, Orange, CA, USA) dual-cured cement. This research investigated the following materials: IPS e.max ZirCAD (yttria-stabilized zirconia polycrystal, Y-TZP; Ivoclar Vivadent, Schaan, Liechtenstein), as a high-strength ceramic with high values of flexural strength and fracture toughness thanks to the crystalline structure [15, 16]; E max CAD (lithium disilicate glass-ceramics; Ivoclar Vivadent), which has a needle-like crystal structure that offers excellent strength and durability as well as outstanding optical properties [17]; and Vita Enamic® (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany), as the first hybrid dental ceramic with a dual-network structure belonging to the polymer-infiltrated ceramic network (PICN) group, where one network is a ceramic material (feldspar, 86 wt%) and the other is a polymer (commonly used methacrylates for dental applications, 14 wt% [18, 19, 20]. Hereinafter, the terms Z-model (Z samples; zirconia), L-model (L samples; e.max), and E-model (E samples; Enamic) will be used due to easier overview. Each group consisted of three different ceramics, thus the total of nine specimens with an implant immersed in the PMMA during the hardening process were manufactured in accordance with the standardized protocol presented in a recently published research [12]. Immediately after initial preparing and spraying (coating), the models were tested on a H10K-S UTM testing machine (Tinius Olsen TMC, Horsham, PA, USA) with a 5 kN load cell, as described in previous studies. The DIC method was used to visualize the strain field in the loaded models. As previously said, the loading speed was 0.1 mm/minute, while the stroke limit was set to 1 mm. We used the force intensities of 100 N, 300 N, and 500 N, respectively, in accordance with the literature data [19]. This was an experimental compressive loading with a gradual increase in the intensity of the applied vertical load. Of the total number of the samples/specimens (n = 9), three representative figures (virtual models) obtained by the software data processing were selected and used to present different stages of the vertically loaded Z, L, and E samples. Strain fields were observed on surfaces 2 mm away from the vertical axis of the implant body. Regions of interest were considered to be surfaces that surrounded the implant body in a projection of the section line, presented in all the figures. In order to facilitate the interpretation of the results, we divided the region of interest into three parts: the cervical region (CR), the middle region (MR), and the apical region (AR).

The following analyses for nine samples (three in each group) were conducted:

- Two-way ANOVA was used in order to examine the differences in the effect of the type of samples, region of interest, and their mutual interaction on the strain values in the sample. The strain values induced by the different ceramic material and strain values within the regions of interest were compared using two-way ANOVA. Significance level (α) was set to 0.05. ($p < 0.05$). All comparisons and calculations were made using the R Stats Package (Software R, Vienna, Austria).
- The post hoc t-test with Bonferroni correction. The post hoc t-test can compare only two strain values at a time.

RESULTS

The relationship between sample type, region of interest, and strain values is displayed in the interaction plot (Figure 1). Comparing all nine samples, the maximum strain (peak) was observed in the ARs and corresponds to the average strain values of 0.30–0.35%, while the minimum strain (0.10–0.15%) was detected in the middle third of the visualized samples (Figure 1). Additionally, the maximum strain was detected in Z samples, while the minimum strain was induced during loading of E samples.

Significant differences in strain values between samples with different specimens were detected ($p = 0.000$). This suggests that strain values were dependent from the type of crown material. Strain values were also affected by the region of interest ($p = 0.000$). The application of two-way ANOVA enabled the testing of interaction effect between two independent variables, the crown material and the region of interest, where a significant difference was found ($p = 0.046$). This indicates that strain values were also influenced by different combinations of material type and region of interest. The highest strain values were found for Z (0.383 ± 0.015) in the AR, and the lowest for E (0.070 ± 0.026) in the MR (Table 1).

The loading of the Z and L samples showed significant differences between all analyzed segments of the region of interest, including the CR, MR, and AR segment ($p < 0.001$). Statistical significance between the MR and CR was set at $p < 0.01$ when L samples were loaded (Table 2). Vertically loaded E samples showed significant differences between the CR and AR, and the MR and AR ($p < 0.001$), while the statistical significance for the MR and CR was set at $p < 0.05$. In the AR, significant difference was noticed between samples Z and E ($p < 0.01$, Table 3). The MR showed significant differences in strain between samples Z and E ($p < 0.001$), Z and L ($p < 0.01$). In the CR, a significant difference was noticed between samples Z and E, and Z and L ($p < 0.01$).

Three types of DIC representative virtual models showed surface strain quantitatively determined by the scales within the DIC figures. Sample surface of the representative software models (virtual models) presented in Figures 2, 3, and 4 generated strain fields during axial loading conditions characterized by gradually increasing the intensity of the strain, which was manifested through color changing from dark blue through green to yellow [10]. Experimental strain field was analyzed using vertical section, as shown in Figures 2, 3, and 4. Section length was around 10 mm. Strain of interest was "on" and "around" the section lines, practically around the implant body. As it can be seen in Figures 2, 3, and 4, the maximum strain was detected in the AR and CR. The lowest strain detected in the region of interest was 0.04%, while the highest strain was 0.40 % for the Z-model (Figure 2). Thus, the Z-model showed higher overall strain than the L-model (Figure 3) or the E-model (Figure 4), where an insignificant strain during the first stage related to a load of 100 N was noticed. Section lines showed the maximum strain in the AR (4%), although the E-model reached only 2.8% even when loaded with 500 N

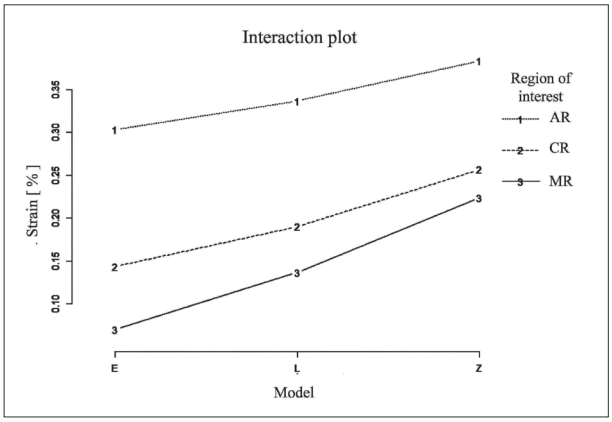

Figure 1. Interaction plot

CR – cervical region; MR – middle region; AR – apical region

Table 1. Means and standard deviations of von Mises strain values for different experimental models (all-ceramics) and regions of interest

Model	Region of interest					
	CR		MR		AR	
	Mean	SD	Mean	SD	Mean	SD
Z	0.257	0.015	0.223	0.015	0.383	0.015
L	0.190	0.010	0.137	0.015	0.337	0.021
E	0.143	0.025	0.070	0.026	0.303	0.015

CR – cervical region; MR – middle region; AR – apical region; Z – Z-model, zirconia; L – L-model, e.max; E – E-model, Enamic

Table 2. Mean values (SD), and significance between locations of interest for identical specimens

Model	Region of interest			p
	CR	MR	AR	
Z	0.26 (0.02)	/	0.38 (0.02)	< 0.001[a]
E	0.14 (0.03)	/	0.3 (0.02)	< 0.001
L	0.19 (0.01)	/	0.37 (0.02)	< 0.001
Z	0.26 (0.02)	0.22 (0.02)	/	> 0.05
E	0.14 (0.03)	0.07 (0.03)	/	< 0.05[b]
L	0.19 (0.01)	0.14 (0.02)	/	< 0.01
Z	/	0.22 (0.02)	0.38 (0.02)	< 0.001
E	/	0.07 (0.03)	0.3 (0.02)	< 0.001
L	/	0.14 (0.02)	0.37 (0.02)	< 0.001[c]

CR – cervical region; MR – middle region; AR – apical region; Z – Z-model, zirconia; L – L-model, e.max; E – E-model, Enamic;
[a]significant difference between CR and AR location of interests, for specimens Z;
[b]significant difference between CR and MR location of interest, for specimens E;
[c]significant difference between MR and AR location of interest, for specimens L

Table 3. Mean values (SD), and significance between specimens and for identical locations of interest

Region of interest	Model			p
	Z	E	L	
CR	0.26 (0.02)	/	0.19 (0.01)	< 0.01[d]
AR	0.38 (0.02)	/	0.37 (0.02)	< 0.05
MR	0.22 (0.02)	/	0.14 (0.02)	< 0.01
CR	0.26 (0.02)	0.14 (0.03)	/	< 0.01
AR	0.38 (0.02)	0.3 (0.02)	/	< 0.01[e]
MR	0.22 (0.02)	0.07 (0.03)	/	< 0.001
CR	/	0.14 (0.03)	0.19 (0.01)	< 0.05
AR	/	0.3 (0.02)	0.37 (0.02)	< 0.05
MR	/	0.07 (0.03)	0.14 (0.02)	> 0.05[f]

CR – cervical region; MR – middle region; AR – apical region; Z – Z-model, zirconia; L – L-model, e.max; E – E-model, Enamic;
[d]significant difference between Z and L specimens, for location of interest CR;
[e]significant difference between Z and E specimens, for location of interest AR;
[f]non-significant difference between E and L specimens, for location of interest MR

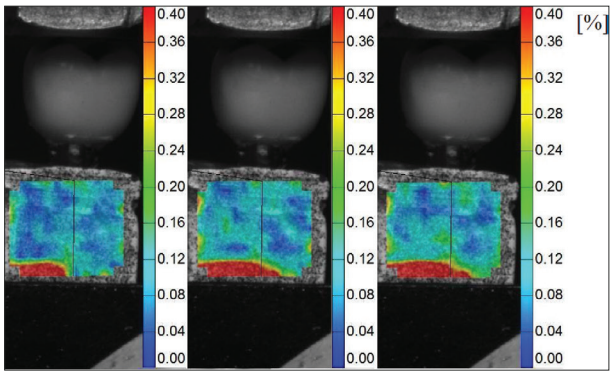

Figure 2. Strain in the Z-model visualized during vertical loading conditions

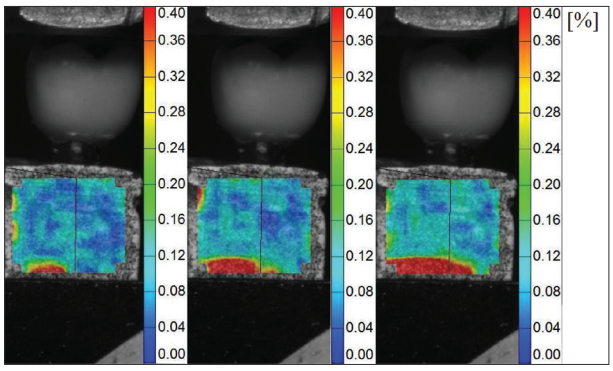

Figure 3. Strain in the L-model visualized during vertical loading conditions

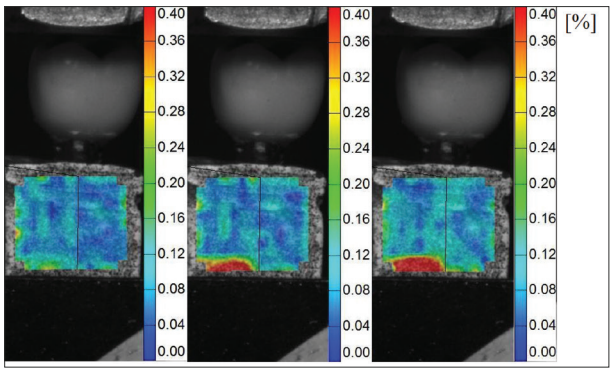

Figure 4. Strain in the E-model visualized during vertical loading conditions

(Figure 4). According to the software data processing, the E-model strained to 0.16%, the L-model to 0.2%, while the Z-model to at least 0.24% in the CR.

DISCUSSION

The study is a preliminary technical report regarding mechanical testing of three types of ceramic systems placed *in situ* on custom-made PMMA samples with immersed dental implants. Actually, all samples were fabricated of the same type of the Straumann® implants/PMMA and the only difference between the samples were different types of all-ceramics. The study was conducted to find which ceramic induced the highest strain in the PMMA block during occlusal loading conditions. It was found that Vita Enamic® (the E-model, Figure 4) induced the lowest strain compared to the others. The presented *in vitro* experiments included a minimum of three identical models of each specimen (Z, L, and E) and showed significant results. Nevertheless, further results will be assessed and argued after examinations on a large number of samples prepared in the same way as presented in this report. DIC showed an ability to measure strain in PMMA induced by the loaded all ceramic crowns. Knowing the fact that the DIC is a surface method and that desirable thickness of bone surrounding an implant is at least 2 mm, strain field was observed on surfaces 2 mm away from the vertical axis of the implant body. This thickness was enough to describe the strain change in PMMA around implant (peri-implant) [20].

The results acquired from the Aramis system (GOM GmbH, Braunschweig, Germany), were sorted in three groups of samples and three groups of interest locations. Ceramics, as the part of the samples and locations of interest within tested models presented factors which caused different values of strain of loaded samples. Their mutual effect on a sample was presented in the interaction plot where the connection between experimental results was visualized. Although strain varied significantly between locations of interest, ceramic-material's effect was also noticed. Namely, samples with zirconia showed the highest strain for every part of interest, including CR, MR, and AR. Enamic samples displayed the lowest strain for all segments of interest. As a hybrid material with PICN, Vita Enamic® includes the best properties of ceramic and composite materials. Additionally, E max CAD crown induced less strain in the L-model than IPS e.max ZirCAD crown induced in the Z-model. The results of this study are consistent with previous findings, where using softer (lower rigidity) crown material reduced the stresses generated on the jaw bone (cortical and spongy). This type of material absorbs more energy from the applied load, and transfers less energy to the following parts of the system (implant–abutment complex and bones) [21]. Particularly, Z specimens had much higher modulus of elasticity (13 GPa) value than E (30 GPa) and/or L specimens (95 ± 5 GPa) [3, 22, 23, 24]. Thus, higher amortization of the vertical loads and lower values of strain in the E model were observed [6, 7, 8].

Strain for different types of samples and different segments of interest was compared using two-way ANOVA, employed to determine whether there was statistical significance in differences between the tested groups. All three ceramic types and locations of interest showed significant influence. Significant differences in strain values existed between three groups of materials, and also in three different regions of interest of the measured surfaces. Although ANOVA revealed statistically significant differences between the type of the strained sample, the region of interest, and the interaction of these two factors, this analysis could not point out the differences between these two factors. Thus, additional post hoc t-test was introduced to reveal a statistical significance between observed variables and to find out where these differences actually occurred.

In order to provide valid comparison and to reduce type I error, the conservative Bonferroni correction was applied. Therefore, all three null hypotheses were rejected and alternative ones were adopted, which state that the strain depended on the ceramic material used and on the location of interest. Also, there was an interaction between ceramics and the region of interest related to strain values. However, the strain values for Z-models were quite similar in the CR and the MR ($p > 0.05$). Furthermore, no significant difference between the E- and the L-model was found considering MR.

The results of this study are consistent with previous reports, where the highest strain was registered in the AR, while the lowest one was observed in the MR [11, 12, 13]. This could lead to the conclusion that the MR of all samples was less sensitive to changes in material composition when compared to CR and AR.

It seems that prosthetic failure was prevented, considering that all ceramics withstood occlusal forces of up to 500 N without breaking, during static loading conditions due to their fracture toughness Z(5.5):L(2.5):E(1.5) MPam [22–25]. Previous researches found that zirconia is the strongest and toughest of all dental ceramics, with superior mechanical properties compared to the glass ceramics (IPS e.max) and hybrid ceramics (Vita Enamic®) [26]. Zirconia belongs to the group of the highest strength ceramics, with outstanding mechanical properties corresponding to its crystalline structure [27]. Flexure strength of zirconia is more than twice as high than that of IPS e.max (glass-ceramics), and even more so when compared to Vita Enamic® [28]. The dominant ceramic network structure supports toughness in Vita Enamic®, while the reinforcing polymer network structure provides viscoelasticity. In this study, a ceramic, like a medium, underwent stress generated by vertical loading with consequent strain detected in the PMMA block. Thus, PMMA indirectly reacted to the implant-supported crown loading. Registered strain actually depends on the strength of the applied ceramics and showed the highest values in the Z-model. Unlike zirconia, Vita Enamic® is, with respect to the elastic modulus, closer to human tooth structure values [6, 7, 8]. As a hybrid material with a PICN, Vita Enamic® includes the best properties of ceramic and composite materials. Composite portion of this material showed higher deformation, which reduces the probability of a spontaneous fracture but it can also reduce the hardness of the ceramic itself and accumulate high percentage of strain in the PICN structure. This has a more beneficial effect on the underlying system of supporting structures, which is actually PMMA in this study.

CONCLUSION

The study determined, evaluated, and visualized surface strain generated in all ceramic samples subjected to vertical loading conditions employing the DIC as a powerful tool for strain analysis. Standardization of the experiment was achieved through using identical PMMA and Straumann® implants for the fabrication of all samples to be tested. Based on the objective of this study and set hypotheses, the following conclusions were derived:

- Mean strain values vary between different types of samples and depend on specimens – all-ceramic crowns. Three viable compositions of all-ceramics transferred different portion of occlusal load over implants, thus generating different strain in PMMA. This fact favors one ceramic over others from the biomechanical viewpoint due to the composition of the ceramic matrix, which may affect potential deterioration of the surrounding supportive structure, in this case PMMA. Furthermore, this gives rise to a possibility of different consequent therapeutic effects on implant-supported restorations.
- A correlation between the mean strain values the and region of interest was registered. Strain was not equally distributed through the region of interest. While an obvious maximum strain was detected in the apical direction, a large portion of strain showed marginal direction.
- Interaction between the specimen and the region of interest was noted. This indicates that all ceramic crowns affect implant–bone interface during vertical loading conditions.

The minimum strain was registered below the Vita Enamic® material, while the maximum strain was found in the samples with zirconia crowns. However, future investigations, with numerous samples will be conducted by employing nondestructive methods, such as the atomic force microscopy, to obtain detailed surface characteristic information and surface quality of tested materials. Also, further clinical studies are necessary to better understand the real biomechanical behavior and interactions of these biomaterials.

Conflict of interest: None declared.

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Анализа деформација у узорцима састављеним од керамичких система применом методе дигиталне корелације слика

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САЖЕТАК

Увод/Циљ Студија је спроведена да идентификује максималну деформацију произведену у узорцима састављеним од полиметилметакрилата, Штрауман® имплантата и три врсте керамичких система.

Методе Коришћене су три врсте експерименталних модела изложених спољашњем оптерећењу од 100 *N*, 300 *N* и 500 *N* и анализираних уз помоћ метода корелације дигиталних слика. Модели су били састављени од итријум-цирконије, е. макс. литијум дисиликатне и хибридних керамика Вита енамик®, постављених на цилиндричне денталне имплантантне системе Штрауман® (4 × 10 *mm*) са абатментима под правим углом.

Резултати Значајне разлике су откривене у вредностима деформација између узорака са различитим керамичким круницама ($p = 0,000$). Ово подразумева да су вредности деформација зависне од типа керамичког материјала. Вредности деформација су зависне и од региона интереса ($p = 0,000$). Примена АНОВА теста је омогућила да се уочи интеракција између независних варијабли, метеријала керамичких круна и региона од интереса, где је такође нађена статистички значајна разлика ($p = 0,046$). Ова чињеница указује на то да вредности деформација зависе од различите комбинације типа керамичког материјала и региона интереса. Највеће вредности деформација су нађене на моделу *Z* (0,383 ± 0,015) у апикалном региону, док су најмање вредности деформација нађене на моделу *E* (0,303 ± 0,015) у региону средње трећине.

Закључак Извештај је показао максималне деформације у апикалним и маргиналним правцима. Када се разматрају различите врсте керамика, најмање деформације су примећене испод круна Вита енамик®, док је највећа деформација пронађена у узорцима са крунама итријум-цирконија.

Кључне речи: керамички системи; деформација; ПММА

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Assessment of reliability and validity of Montenegrin version of the oral health impact profile for use among the elderly in Montenegro

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SUMMARY

Introduction/Objective The quality of life of elderly individuals has an active function in oral health; it is of great importance to learn that elders over the age of 65 years demonstrate an increase in seeking dental services. Oral Health Impact Profile-14 (OHIP-14) is especially suitable for use in the elderly. The aim of this study is to examine the reliability and validity of OHIP-14 in the Montenegrin population aged 65 and over and to determine the influence of oral health on the quality of their life.

Methods The research was conducted from September to December 2016 in the central region of Montenegro, at the Medical University in Podgorica and in the nursing homes of the elderly. The study covered 170 individuals, both sexes, with an average age of 72.32 ± 6.85. The research instrument is OHIP-14 index. Standard statistical tests were used. The statistical significance level is 0.05.

Results The OHIP-14is linguistically and culturally adapted for the Montenegrin population. The value of the Cronbach Alpha Index is 0.892. The relationship between correlations for individual issues and total correlations ranges from 0.21 to 0.69. The value of OHIP-14 is 19.24 ± 7.49. Listed by domains: functional constraints 3.31 ± 1.75; physical pain 4.19 ± 1.31; psychological discomfort 2.52 ± 1.46; physical fitness 4.38 ± 1.40; mental incompetence 1.42 ± 1.23; social incapacity 1.18 ± 1.27 and handicap 2.21 ± 1.32.

Conclusion The OHIP-14 index is reliable and valid and is recommended for use in the Montenegrin-speaking area, for the elderly. There is a significant impact of oral health on the quality of life of the elderly in the central part of Montenegro.

Keywords: quality of life; elderly; Montenegro

INTRODUCTION

The development of medicine and science in general has led to a prolonged life span. Demographers predict that by 2060 the average age of citizens of the European Union will be 47.2 years. In the next 40 years, people over 65 will make up nearly 30% of the European Union's population [1, 2]. Increased care for the aging, promotion, and implementation of the concept of "active aging" aims to contribute to the improvement of health, quality of life, and attainment of aging [3]. It is necessary to know the state of oral health of the elderly, and the impact it has on the quality of life, in order to be able to plan and organize dental care, as the ever-increasing number of individuals will be in need of that service in the future.

The health of the mouth and teeth is not considered only as the absence of the disease, but also the functional, psychological, and social aspect of oral diseases is examined.

This is fully in line with the definition of health of the World Health Organization and the definition of oral health [4, 5]. New definition of oral health: "Oral health is multifaceted and includes the ability to speak, smile, smell, taste, touch, chew, swallow, and convey a range of emotions through facial expressions with confidence and without pain, discomfort, and disease of the craniofacial complex" [5, 6].

The first authors who began to examine the psychosocial aspect of oral health were Cohen and Jago in the 1970s. During the 1980s and 1990s, Reisine, Bailit, Sheiham, Croog, Roseenberg and many others continued the trials. Today, there are a vast number of clinically verified indices, and each day they are improving postures and creating new indexes [7, 8, 9].

The original version of the Oral Health Impact Profile (OHIP) has 49 questions (OHIP-49) [10], but in a large number of researches, there are uses of a reduced form of OHIP, with 14 questions. Slade first tested the reduced version in 1997, and then by Locker and Allen in 2002 [11, 12, 13]. Slade had shown that the shorter version has the same reliability and validity as the original version, and due to a smaller number of questions, it is particularly suitable for the research of the quality of life in the elderly [11]. To date, OHIP-14 has been translated into a number of languages and has been found in many countries around the world [14–23]. It is a comprehensive and multi-dimensional index

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that covers all aspects of physical health to the psychological and social sphere of life. It was designed so that it could be applied to people with different general characteristics (education, occupation, culture, social status, and other characteristics). OHIP-14 has experienced linguistic and cultural adaptation, clinical verification, and verification in countries such as Montenegro and its surroundings [24–27].

The aim of this study was to examine the reliability and validity of OHIP-14 in the Montenegrin population aged 65 and over and to determine the influence of oral health on the quality of their life in the central part of Montenegro.

METHODS

Prior to the realization of the research, the study was approved by the Ethical Committee of the Faculty of Medicine of the University of Montenegro in Podgorica. Respondents who participated in the research were previously informed and received their written consent for participation in the research. A research plan had been developed. A dentist who is a specialist in the field of dental prosthetics performed the examination.

The research was conducted from September to December 2016 in the central part of Montenegro, at the Medical University in Podgorica and in the nursing homes for the elderly "Ljubav Spaja" and "Nana" in Spuž and Danilovgrad.

Research sample

The study conducted covered 170 people. The average age of the respondents was 72.32 ± 6.85 (65–91). The sample accounted for 5% of the total population aged 65 and over, living in the central part of Montenegro. The research includes:

1. Persons aged 65 and over who have appeared for an examination at the Faculty of Medicine in Podgorica – Study program for dentistry, on specific days (Mondays and Wednesdays) from September to December 2016.
2. The beneficiaries of the services of nursing homes "Nana" and "Ljubav Spaja" in Spuž and Danilovgrad, whose state of general and mental health allowed them to be interviewed.

Research instruments

To determine the impact of oral health on the quality of life, the OHIP-14 index was used [11].

The OHIP-14 questionnaire consists of 14 questions. Each response is scored at a value of 0–4, using the Likert scale with five responses, depending on the extent to which the patient is affected by the problem. Over the past 12 months, according to one's own assessment: (0 – no problems at all; 1 – have problems; 2 – often have problems; 3 – very often have problems; 4 – constantly have problems). The maximum possible number of points is 56. The higher the score was, the higher the negative impact of oral health on the quality of life of the respondents.

Since OHIP-14 was not applied in the Montenegrin population, it was first necessary to examine its reliability and validity.

The original OHIP-14 questionnaire was first translated using a two-way (back) translation (from English to Montenegrin and back). Two licensed interpreters translated the questionnaire from English into Montenegrin independently of each other. Then a person who was not familiar with the contents of the original text of the questionnaire made a back translation from Montenegrin to English (the person was a good connoisseur of both languages). It was taken into consideration that the essence of the issue was preserved and that the translation was simultaneously adapted to the Montenegrin language, the mentality, and culture of the population.

The first 30 respondents of this study who completed the questionnaire (independently or with the help of the dentist) understood the significance of all 14 questions and expressed the degree to which certain problems were expressed.

The reliability of the OHIP-14 questionnaire was established using the Cronbach Alpha test, which is standardly used to test the reliability of this and similar questionnaires. The reliability of the questionnaire was assessed by examining the internal consistency (homogeneity) of the answers from the questionnaire. In doing so, testing has been completed in three ways:

1. Omitting individual items (questions), while tracking changes in the Cronbach Alpha value;
2. By following the correlations between items interacting with each other;
3. Calculating the total correlations for all items.

For the purposes of further investigation, a regression analysis was performed where the dependent variable was the total OHIP-14 result, and the independent variables were sub-bases OHIP-14.

The OHIP-14 instrument has been tested according to various types of validity. First, the validity of the form and content of the questions was verified in the pilot study. The validity of the OHIP-14 as an instrument was tested by correlating (and examining the existence of a statistically significant difference) of OHIP-14 values depending on the specific characteristics of the respondents (age of the respondent, sex, presence and type of dental remuneration, education and occupation of the respondents).

In statistical data processing, structural validity was tested using Student's t-test for two independent samples, one-way ANOVA with Bonferroni or Tamhane T2 after hoc tests. The homogeneity dispersion was checked by Leven test. The level of statistical significance was 0.05.

RESULTS

The study conducted involved 170 people, the average age was 72.32 ± 6.85, of which: 104 (61.17%) subjects aged 65–74, 53 (31.17%) respondents aged 75–84, and 13 (7.6%) subjects aged 85 and older. Of the 170 respondents, there were 89 (52.35%) female examinees and 81 (47.64%) male

Table 1. Correlation between items (questions) of OHIP-14

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.00													
2	-0.09	1.00												
3	0.41#	0.10	1.00											
4	0.45#	0.15*	0.10	1.00										
5	0.43#	-0.06	0.21#	0.33#	1.00									
6	0.36#	-0.01	0.18*	0.27#	0.42#	1.00								
7	0.37#	0.18*	0.11	0.77#	0.41#	0.22#	1.00							
8	0.18*	0.06	0.15*	0.38#	-0.06	0.28#	0.21#	1.00						
9	0.38#	0.04	0.05	0.47#	0.39#	0.34#	0.54#	0.11	1.00					
10	0.47#	-0.08	0.12	0.44#	0.41#	0.27#	0.42#	0.14	0.57#	1.00				
11	0.47#	-0.05	0.13	0.45#	0.51#	0.29#	0.44#	0.08	0.52#	0.75#	1.00			
12	0.46#	-0.11	0.12	0.43#	0.45#	0.30#	0.48#	0.08	0.57#	0.61#	0.75#	1.00		
13	0.54#	-0.05	0.25#	0.49#	0.46#	0.37#	0.42#	0.26#	0.46#	0.48#	0.48#	0.51#	1.00	
14	0.43#	0.08	0.12	0.47#	0.44#	0.24#	0.57#	0.06	0.52#	0.44#	0.48#	0.55#	0.56#	1.00

1–14 – questions from the OHIP-14 questionnaire;
*p < 0.05;
#p < 0.01

examinees. The structure of the respondents according to the level of education: the majority of respondents 64 (37.64%) have secondary education, 39 (22.94%) have higher education (university), 27 (15.88%) have post-secondary education (college), 32 (18.82%) with elementary education, while eight (4.70%) are without education. The structure of respondents by occupation (prior to retirement) demonstrated that the majority, 46 (27.05%) of respondents were in the field of service activities, 34 (20%) were occupied with production and 33 (19.41%) in the field of law and economics. There were a smaller number of respondents in the field of education 18 (10.58%) and lastly, there were 14 (8.23%) health workers. The condition of oral health of the respondents who participated in the research was not satisfactory. Out of the 170 people examined, 79 (46.47%) were without teeth. It was found that in 83 (48.82%) people there were mobile dental prostheses in both jaws. Of the respondents examined, 34 (20%) have mobile dental prostheses in one jaw, and teeth in the opposite jaw are not reimbursed. Denture strings replaced with fixed dental prostheses are present in 16 (9.41%) persons. The respondents who had one jaw mobile and the other fixed denture were 11 (5.88%). In 115 (67.65%) subjects, it was estimated that there was a need for rapid treatment, which mainly relates to the necessity of making new dental prosthesis. There was no need for emergency dental intervention in any of the respondents.

The Oral Health Impact Profile-14 reliability analysis

The value of the Cronbach Alpha index, derived from the correlation matrix, is 0.892. The internal consistency of OHIP-14 was first assessed by the analysis of the correlation between the items (questions) (Table 1). Differences in the value of coefficients have shown that no item is superfluous and it is necessary that all questions remain in the questionnaire.

Analyzing results in case of removal of individual items (questions) supports the inclusion of all questions that are in the original questionnaire. The relationship between correlations for individual items (questions) and total, correlations ranges 0.21–0.69. The total correlation analysis (correlation between one item and all others) showed that all coefficients are above the minimum recommended value (0.20), which is necessary to include the question in the questionnaire (Table 2).

Table 2. Values of Cronbach Alpha coefficients and degree of correlation expressed on issues from questions 1–14

Questions	Correlation of questions/ total correlation	Value of Cronbach's alpha index when a question is omitted
1	0.611	0.835
2	0.211	0.873
3	0.285	0.850
4	0.690	0.826
5	0.511	0.83
6	0.425	0.843
7	0.666	0.828
8	0.250	0.855
9	0.627	0.833
10	0.628	0.835
11	0.665	0.833
12	0.646	0.834
13	0.678	0.830
14	0.635	0.834

1–14 – questions from the Oral Health Impact Profile-14 questionnaire

All of the above points to the high reliability of the OHIP-14 questionnaires and recommends it to be used in the Montenegrin-speaking area, in older persons.

The Oral Health Impact Profile-14 structural design analysis

The validity of the OHIP-14 as an instrument was tested by correlating and examining the existence of a statistically significant difference in the value of OHIP-14 according to certain characteristics of the respondents.

Monitoring of the Oral Health Impact Profile-14 value in relation to sex, age, education and occupation of respondents (prior to retirement)

Respondents aged 75 and older have a statistically significantly higher OHIP-14 value (20.76 ± 7.39) compared to patients aged 65–74 (18.27 ± 7.43) (Student's t-test for two independent samples, t = -2.132; p = 0.034) (Table 3).

In the male sex ratio, the OHIP-14 value is (19.81 ± 8.53) higher than for female respondents (18.54 ± 6.15), but there is no statistically significant difference between these values (Student's t-test for two independent samples, t = 1.252; p = 0.213) (Table 3).

Respondents who do not have education or have primary education have higher OHIP-14 values (20.50 ± 6.87) compared to those with secondary education (18.78 ± 6.93), post-secondary and higher education (18.73 ± 7, 64). There is no statistically significant difference between these values (ANOVA, F = 0.391; p = 0.815) (Table 3).

There is no statistically significant difference in the value of OHIP-14 according to the respondents' occupations (ANOVA, F = 1.072; p = 0.384). The highest value of OHIP-14 is for the respondents who worked in production (20.29 ± 7.50). The lowest value of OHIP-14 is for educators and health workers (18.38 ± 8.35) (Table 3).

Monitoring of the Oral Health Impact Profile-14 value in relation to the type of dental prosthesis

There is a statistically significant difference in OHIP-14 values relative to the type of dental prosthesis (ANOVA; F = 111.892; p < 0.001). The Leven test indicates that dispersions may be considered homogeneous (p = 0.267) (Table 4).

Table 4. Values of OHIP-14 according to the type of denture

Type of (I) denture	n	Value of OHIP-14	Type of (J) denture	Difference of mean value (I and J)	p-value
NN	27	31.66 ± 3	FN	24.479*	< 0.001
			2MN	14.438*	< 0.001
			NZ	11.108*	< 0.001
			MN + FN	14.567*	< 0.001
FN	16	7.19 ± 4.25	NN	-24.479*	< 0.001
			2MN	-10.041*	< 0.001
			NZ	-13.371*	< 0.001
			MN + FN	-9.913*	< 0.001
2MN	82	17.17 ± 5.01	NN	-14.438*	< 0.001
			FN	10.041*	< 0.001
			NZ	-3.330*	< 0.001
			MN + FN	0.129	1.000
NZ	34	20.55 ± 3.38	NN	-11.108*	< 0.001
			FN	13.371*	< 0.001
			2MN	3.330*	0.001
			MN + FN	3.459	0.156
MN+FN	11	17.54 ± 4.11	NN	-14.567*	< 0.001
			FN	9.913*	< 0.001
			2MN	-0.129	1.000
			NZ	-3.459	0.156

NN – no denture; FN – fixed denture; 2MN – mobile denture in both jaws; NZ – incompletely replaced tooth (due to mobile denture in one jaw, no denture in the other); MN + FN – mobile denture in one jaw, fixed denture in the other

1. Persons who are partially or completely free of natural teeth and do not have remuneration have a statistically significantly higher OHIP-14 value than:
   – a person with a fixed dental prosthesis (Bonferroni test, p < 0.0001),
   – a person with a mobile prosthesis in both jaws (Bonferroni test, p < 0.0001),

Table 3. Values of the OHIP according to sex, age, education, and occupation of the respondents (before retirement)

Structure of respondents	n	%	Mean value OHIP-14	± SD	Statistical test and statistical significance
Sex					
Male	81	47.64	19.81	8.53	Student t-test for two independent samples; t = 1.252; p = 0.213
Female	89	52.35	18.54	6.15	
Age					
65–74 years	104	61.17	18.27	7.43	Student t-test for two independent samples; t = -2.132; p = 0.034**
75 years and older	66	38.77	20.76	7.39	
Education					
Uneducated	8	4.7	20.50	6.87	ANOVA test F = 0.391; p = 0.815
Elementary education		18.82			
Secondary education	64	37.64	18.78	6.93	
Post-secondary (college)	27	15.88	18.73	7.64	
Higher education (university)	39	22.94			
Occupation					
Service activities	46	27.05	19.09	7.29	ANOVA test F = 1.072; p = 0.384
Production	34	20	20.29	7.50	
Field of law & economy	33	19.41	19.96	6.96	
Field of education	18	10.58	18.38	8.35	
Health workers	14	8.23			

**Old significant difference

– a person with incompletely replaced teeth (there is a mobile denture in one jaw, and in the other lost teeth are not denture) (Bonferroni test, $p < 0.0001$),
– persons with one jaw fixed denture, and in the other mobile (Bonferroni test, $p < 0.0001$).

2. Persons with fixed dental prosthesis have a statistically significantly lower OHIP value than:
   – a person with mobile dental prosthesis in both jaws (Bonferroni test, $p < 0.0001$),
   – a person with incomplete restored tooth (Bonferroni test, $p < 0.0001$),
   – persons with one jaw fixed denture, and mobile in the other (Bonferroni test, $p < 0.0001$).
3. Persons with mobile denture in both jaws have a statistically significantly lower OHIP value of:
   – a person with incompletely replaced teeth (in one jaw they have mobile denture and in the other they have no natural teeth or dental prosthesis) (Bonferroni test, $p = 0.001$).

The impact of oral health on the quality of life of the elderly

The value of OHIP-14 in this study is 19.24 ± 7.49 (min. 0, max. 37). The OHIP-14 values expressed in terms of domains are as follows: functional limits 3.31 ± 1.75, physical pain 4.19 ± 1.31, psychological discomfort 2.52 ± 1.46, physical fitness 4.38 ± 1.40, psychological incompetence 1.42 ± 1.23, social incapacity 1.18 ± 1.27, and handicap 2.21 ± 1.32.

The majority of people (91%) expressed difficulties in the field of physical incapacity, eating disorders (82% answered that "very often" or "constantly" change their diet regime due to mouth, teeth, or dental problems). Physical pain has been present in 90% of people in the last 12 months (74% of respondents "very often" or "constantly" avoids certain foods). Functional constraints were 78% (40% of respondents "often" and 36% "very often" or "constantly" have a feeling of taste change due to the condition of the mouth, teeth, and compensation). The impact is least pronounced in the domains of psychological incompetence (56%) and social incompetence (51%) (Table 5).

DISCUSSION

The study of the impact of oral health on the quality of life was first carried out in Montenegro. The existence of the OHIP-14 translation into the languages of the states in the region of Montenegro has greatly facilitated the process of linguistic and cultural adjustment of the original text of the OHIP-14.

The Serbian version of this index has 13 questions, because the fifth question was left out (Have you been self-conscious because of your mouth or dentures?) [24]. In the pilot study, the authors estimated that the translation of this question was such that the issue was not sufficiently understandable for a significant number of respondents and that the questionnaire had a sufficient number of other questions from the psychosocial sphere. Croatian authors translated the fifth question from OHIP-14 questionnaires differently and put it in a questionnaire [24]. In order to adjust the spirit of the language in the Japanese version, a further 14 questions have been added to the fifth. It is considered that the structure of the questionnaire allows such changes, since the overall score is not crucial for the validity of the index [24].

The value of Cronbach alpha coefficient, derived from the correlation matrix, is 0.892 in this study. This is significantly more than the minimum recommended value of 0.70 [13, 14]. This recommends OHIP-14 for use among the Montenegrin population of the elderly.

The values of Cronbach alpha coefficient in studies that also examined the applicability of the OHIP-14 index among the elderly (with similar characteristics of the sample) are listed below. In a study conducted in Jordan, as in the Montenegrin study, the Cronbach alpha coefficient value is 0.89 [17]. In studies conducted in Greece and in Italy, the value of Cronbach's alpha coefficient was 0.90 [15, 18]. In a study done in Chile, the value of Cronbach's

Table 5. Expression of the influence of oral health on the quality of life

Domains OHIP-14	Quest. OHIP-14	"I had no problems" n (%)	"Rarely" n (%)	"Often" n (%)	"Very often" "Constantly" n (%)	Domains %
Functional constraints	1	76 (46)	43 (25)	18 (10)	33 (19)	78
	2	18 (10)	25 (14)	65 (40)	72 (36)	
Physical pain	3	27 (15)	91 (53)	45 (28)	7 (4)	90
	4	5 (3)	6 (3)	33 (20)	126 (74)	
Psychological discomfort	5	34 (20)	57 (33)	58 (35)	21 (12)	77
	6	41 (24)	78 (45)	40 (29)	11 (6)	
Physical incapacity	7	5 (3)	11 (6)	13 (7)	141 (82)	91
	8	25 (15)	58 (34)	63 (37)	24 (14)	
Psychological incompetence	9	77 (45)	67 (39)	24 (15)	2 (1)	56
	10	70 (41)	81 (47)	18 (11)	1 (1)	
Social incapacity	11	70 (41)	75 (44)	23 (14)	2 (1)	51
	12	95 (55)	59 (34)	15 (10)	1 (1)	
Handicap	13	11 (6)	95 (56)	33 (19)	31 (18)	78
	14	62 (36)	86 (50)	19 (12)	3 (2)	

1–14 – questions from the Oral Health Impact Profile-14 questionnaire

alpha coefficient is 0.91 [19]. In Poland, a study dealing with two General Oral Health Assessment Index (GOHAI) and OHIP-14 indexes showed that the Cronbach alpha coefficient value was 0.89 for GOHAI and 0.97 for OHIP-14 [16]. The value of the Cronbach Alpha coefficient is 0.78, in one of numerous studies in Brazil [20].

The validity of the OHIP-14 index was estimated by correlating the OHIP-14 index values and individual characteristics of the respondents. In the Montenegrin study, patients aged over 75 had a statistically significantly higher OHIP-14 value compared to patients aged 65–74 (20.76 ± 7.39 versus 18.27 ± 7.43). In the elderly, the condition is where the mouth and teeth are worse (more missing teeth) and largely there are mobile dentures older than five years. Such remuneration no longer meets aesthetic, functional, and prophylactic requirements and does not follow changes in the dental system resulting from the physiological aging process.

The study showed that there is a statistically significant difference in the value of OHIP-14, depending on the type of denture. The highest value was observed in persons whose missing teeth were not replaced by dental prostheses. The lower value is in the holder of fixed prostheses in relation to persons with mobile prostheses. In a similar sample study done in Iran (the average age was 67.5 ± 11, a high degree of no teeth, 87.5% without front teeth, 85.6% without side teeth, 31.3% had total dentures and 28.8% of the partial like in the Montenegrin research), it was estimated that OHIP-14 was statistically significantly higher in those who were not prosthetically treated compared to persons who did not require prosthetic treatment (25.75 ± 14.5 according to 21.18 ± 9.8; p = 0.02) [28]. The Polish authors conducted a two-index study (GOHAI and OHIP-14) in 2014 [17]. They concluded that the condition of the teeth, the presence of partial dentures, chewing problems and other problems with the mouth and teeth are significantly related to the values of both indexes. OHIP-14 was significantly higher in patients without teeth (26 ± 15.2) compared to those who had their own teeth (12.5 ± 13). Prosthetically rehabilitated patients were significantly lower (12 ± 12.9) compared to persons without natural teeth and without dental prostheses (22.5 ± 12.9).

In this study, the researchers estimated that the greatest influence of oral health on their quality of life is present in the domains: physical incapacity, physical pain, and functional constraints. The smallest influence is in the domains: psychological disability and social incapacity. If one compares the results of the Montenegrin study with the results of the studies within the region and surrounding countries (Serbia, Macedonia, Croatia, Greece), the results are similar [15, 24–27]. Namely, in the areas of physical incapacity and functional limitations, the respondents expressed the greatest influence. The smallest influence was expressed in the domains of psychological and social incompetence.

Among the questionnaires that are used to examine the effect of oral health on quality of life in the elderly, we decided to use the OHIP-14 in this research because it was previously used in many countries. There are many scientific papers where OHIP-14 was used. We wanted to do research in Montenegro and compare our results with results in the world. We plan a research that will examine the quality of life before and after prosthodontic rehabilitation in the elderly and we will use the GOHAI questionnaire.

There is a limitation of this study in the use of OHIP-14 in middle-aged people of Montenegro. In the future, the impact of oral health on the quality of life among this and other age groups should be examined.

CONCLUSION

The OHIP-14 index is reliable, valid, and recommended for use in Montenegro among the elderly. There is a significant influence of the condition in which the mouth, teeth, and dental compensations are placed on the quality of life of the elderly in the central region of Montenegro. The influence of these conditions is mostly in the areas of physical incapacity, physical pain and functional limitations, and the smallest in the domains of psychological and social incapacity. In order to improve the condition of oral health and the quality of life of the elderly, it is necessary to plan and continuously work on the development of the dental service and on health literacy and education. It would be useful to form geronto-stomatological teams within health institutions and promote the concept of active aging.

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Процена поузданости и валидности црногорске верзије профила утицаја оралног здравља на квалитет живота старих особа у Црној Гори

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САЖЕТАК

Увод/Циљ Квалитет живота старих особа у функцији оралног здравља има велики значај с обзиром на повећање броја корисника стоматолошких услуга старијих од 65 година. *Oral Healt Impact Profile*-14 (*OHIP*-14) нарочито је погодан за примену код старих особа.

Циљ истраживања је да се испита поузданост и валидност *OHIP*-14 код црногорског становништва старости 65 и више година и да утврди утицај оралног здравља на квалитет њиховог живота.

Методе Истраживање је рађено од септембра до децембра 2016. у средишњем региону Црне Горе, на Медицинском факултету у Подгорици и у домовима за стара лица. Истраживањем је обухваћено 170 особа, оба пола, просечне старости 72,32 ± 6,85 година. Инструмент истраживања је индекс *OHIP*-14. Коришћени су стандардни статистички тестови. Ниво статистичке значајности је 0,05.

Резултати Индекс *OHIP*-14 је језички и културолошки прилагођен за црногорско становништво. Вредност индекса Кронбахове алфе износи 0,892. Однос између корелација за поједина питања и укупне корелације креће се од 0,21 до 0,69. Вредност *OHIP*-14 износи 19,24 ± 7,49. Исказано по доменима: функционална ограничења 3,31 ± 1,75; физички бол 4,19 ± 1,31; психолошка нелагодност 2,52 ± 1,46; физичка неспособност 4,38 ± 1,40; психичка неспособност 1,42 ± 1,23; социјална неспособност 1,18 ± 1,27 и хендикеп 2,21 ± 1,32.

Закључак Индекс *OHIP*-14 је поуздан и валидан и препоручује се за употребу на црногорском говорном подручју, код старих особа. Постоји значајан утицај оралног здравља на квалитет живота старих особа у средишњем делу Црне Горе.

Кључне речи: квалитет живота; старе особе; Црна Гора

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Occlusal appliances – an alternative in pain treatment of temporomandibular disorders

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SUMMARY

Introduction/Objective The pain that originates from musculoskeletal structures of the mastication system is one of the symptoms belonging to the category of temporomandibular disorders or temporomandibular dysfunction (TMD).

The objective of the research was to evaluate the effect of therapy with stabilizing occlusal splint in the control of painful symptoms of TMD in comparison with the effect of drug therapy.

Methods Using standard Research Diagnostic Criteria for Temporomandibular Disorders diagnostic protocol proposed by Dworkin and LeResche, a group of 44 patients with painful TMD was included. The patients were divided into three treatment groups by random selection. The first group was treated with stabilization occlusal splint for a period of one month. In the two control groups, therapy with non-steroidal anti-inflammatory drug ibuprofen (Brufen, Mylan, Canonsburg, PA, USA) or a combination therapy of ibuprofen and diazepam, a medication from the benzodiazepine family (Diazepam, Hemofarm, Vršac, Serbia) was carried out over a period of three weeks. In order to assess the effects of the therapy with stabilizing occlusal splint and the drug therapy, before and after the therapy, pain intensity measurements were performed with visual analogue scale and digital pressure algometer.

Results A significant reduction in the intensity of painful symptoms was achieved in all three therapeutic groups. No significant differences in the effectiveness of pain reduction between the proposed therapeutic modalities were noted.

Conclusion The obtained results confirm that the therapy with stabilization occlusal splint is a valid procedure in the reduction of pain in patients with TMD.

Keywords: temporomandibular dysfunction; occlusal splint; pharmacotherapy

INTRODUCTION

Pain in the orofacial region is a signal of tissue damage and complicates most dental procedures. The presence of pain endangers the psycho-physical health and, indirectly, social and working abilities of patients. For the mentioned reasons, the first step in the treatment of various forms of temporomandibular dysfunction (TMD) is the reduction of the intensity of pain and the relaxation of the mastication muscles [1, 2].

In the treatment of patients with signs and symptoms of painful TMD, different therapeutic modalities are used, which should not give negative side effects, nor cause irreversible structural changes in tissue [3]. The concept of therapy with occlusal stabilization splint is based on several therapeutic mechanisms, indirectly taking part in the control of painful symptoms and reducing the intensity of pain [4, 5].

The objective of the study was to examine in parallel the analgesic effect of the occlusal stabilization splint in relation to the effect of drug therapy in the reduction of painful symptoms in individuals with clinically confirmed signs of TMD.

METHODS

The research was conducted as a prospective study involving 44 subjects divided into three treatment groups heterogeneous by sex and age, who came to the Clinic for Prosthodontics, University of Belgrade, with TMD symptoms. A standardized protocol for TMD, proposed by Dworkin and LeResche [6], was used for diagnosing and numerically expressing pain intensity. Respondents were divided into three treatment groups formed by random selection based on the Research Diagnostic Criteria for Temporomandibular Disorders protocol. The first group consisted of 20 patients who received therapy with a stabilization splint (Figures 1 and 2). The remaining 24 respondents were divided into two control groups that had therapy with non-steroidal anti-inflammatory drug ibuprofen (Brufen, Mylan, Canonsburg, PA, USA) or a combination therapy of ibuprofen and diazepam, a medicine from the benzodiazepine family (Diazepam®, Hemofarm, Vršac, Serbia). All three groups were of equal age structure in the range of 25–45 years. The respondents were thoroughly informed about the protocol of the study and gave voluntary consent to participate in the study, which was

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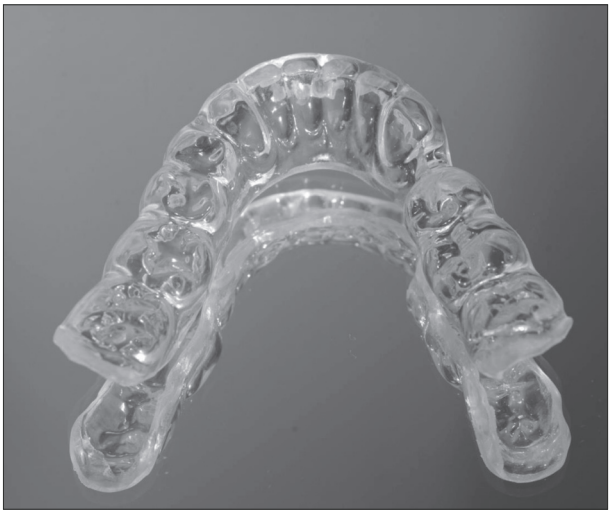

Figure 1. Stabilization splint made of thermoplastic poly-carbonate

approved by the Ethics Commission of the Faculty of Dentistry, University of Belgrade. The chosen methodology was applied to each patient individually.

Algometric measurement was performed in parallel with visual analogue scale (VAS) and digital algorithm. The pain threshold was measured by a digital algometer in the region of *m. masseter* and *m. temporalis*, on both sides. Measuring sites corresponded to palpable painful sites observed during the clinical examination. Painful places were previously marked with an ink pencil.

In order to measure the pressure threshold of the pain, the rubber tip of the algometer-probe was attached to the facial skin in the projection of the painful site, applied by a suitable procedure.

Measurement implied a gradual increase in mechanical pressure to a painful place in the interval of 0.5 N/sec. The respondent was instructed to verbally report the moment of pain. The measurement was repeated three times, with 5-minute pauses between the measurements. The measured force is displayed on the machine's display in newtons (N). The pain threshold was defined as the moment in which the patient's sense of pressure turned into a painful sensation. The pain threshold was calculated as the mean of the two last measurements, of three consecutive measurements.

The pressure measurement was performed at bilaterally symmetrical points. The respondent was informed that the same pressure force was applied on both sides. The intensity of the pain was measured in the same time and space conditions.

In order to minimize the error in algometer measuring, the respondents were asked to avoid consuming alcohol, nicotine, and caffeine on the day of the measurement. The same procedure after the therapy had been administered was applied in all therapeutic groups. A digital algometer (FORSE ONETM FDIX, Wagner Instruments, Greenwich, CT, USA) was used in the research; it has a National Institute of Standards and Technology of the US Department of Commerce certificate, and is registered at the US Patent Office under the number 5,471,885.

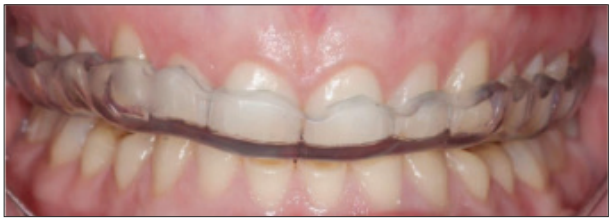

Figure 2. Stabilization occlusal splint as a therapeutic option for pain reduction in patients with temporomandibular disorders

Respondents in the control group were treated with combined therapy of ibuprofen (Brufen, Mylan, 400 mg, twice daily during a 12-hour period, after meals) and diazepam (Diazepam, Hemofarm, 5 mg, one hour before bedtime) over a period of three weeks, or with ibuprofen alone (400 mg, twice daily during a 12-hour period, after meals) during the same time frame. Since benzodiazepines are administered in smaller doses, the hypnotic effect of these drugs was avoided. Diazepam doses were gradually reduced before completion of therapy in order to avoid the recurrence of the disorder symptoms. Applied medicines have the ISO certificate and registration certificate at the Agency for Medicines and Medical Devices of Serbia.

PASW Statistics, Version 18.0 (SPSS Inc., Chicago, IL, USA) software was used for all statistical analysis. The level of statistical significance was set at $p < 0.05$.

RESULTS

The age of subjects with different orofacial pain treatment did not statistically significantly differ among subjects of different therapeutic groups. A statistically significant difference in the incidence of TMD was observed between different sexes. All subjects in the pharmaceutically treated group were female, while in the group treated with the stabilization splint there were 35% men and 65% women (Table 1).

Table 1. Number and demographic characteristics of respondents

Observed parameters		Therapy			p
		Ibuprofen + diazepam	Occlusal splint	Ibuprofen	
Number of respondents		8	20	16	
Age (X ± SD)		44.63 ± 12.56	35.6 ± 10.7	38.5 ± 9.5 4	0.136[a]
Sex n (%)	Male	0 (0%)	7 (35%)	0 (%)	0.007[b,*]
	Female	8 (100%)	13 (65%)	16 (100%)	

*Statistically significant difference;
[a]single-factor analysis of variance;
[b]χ^2 test

Between the analyzed groups treated with different therapeutic approaches, there was no statistically significant difference in the cause of the existing pain. In the treatment group treated with analgesics and sedatives (62.5%), as well as in the group treated with the stabilization splint (55%), the majority of subjects had musculoskeletal dysfunction, while in the group treated only with analgetics the frequency of subjects with joint and musculoskeletal dysfunction was the same (37.5%) (Table 2).

Table 2. Distribution of respondents according to diagnosis in relation to therapy

Diagnosis (disfunction)		Therapy			p
		Ibuprofen + diazepam	Occlusal splint	Ibuprofen	
n (%)	Muscular	1 (12.5%)	2 (10%)	4 (25%)	0.657
	Articular	2 (25%)	7 (35%)	6 (37.5%)	
	Musculo-skeletal	5 (62.5%)	11 (55%)	6 (37.5%)	

Between the analyzed groups, treated with different therapeutic approaches, a statistically significant difference in the values of subjective intensity of pain (VAS) was not noticed before the therapy, nor after it. Between the analyzed therapeutic groups, there was no statistically significant difference in pain intensity with an objectively registered digital algometer (DA), before and after the performed therapy. A statistically significant difference in pain intensity was observed in all treatment groups before and after the therapy, regardless of the chosen treatment method (Table 3).

Table 3. Subjective and objectively assessed intensity of pain before and after the therapy

Pain intensity (X ± SD)	Therapy			p
	Ibuprofen + diazepam	Occlusal splint	Ibuprofen	
VAS′	57.00 ± 21.29	59.05 ± 20.60	59.13 ± 15.61	0.962
VAS″	34.00 ± 18.99	28.55 ± 17.79	34.75 ± 17.73	0.553
VAS′ vs. VAS″	p = 0.001*	p = 0.000*	p = 0.000*	
DA′	10.86 ± 1.81	11.27 ± 3.60	10.53 ± 2.25	0.751
DA″	15.42 ± 2.08	14.55 ± 3.76	15.14 ± 2.59	0.755
DA′ vs. DA″	p = 0.001*	p = 0.000*	p = 0.000*	

VAS′ – pain intensity assessed by visual analog scale before the therapy;
VAS″ – pain intensity assessed by visual analog scale after the therapy;
DA′ – pain intensity assessed by digital algometer before the therapy;
DA″ – pain intensity assessed by digital algometer after the therapy;
*statistically significant difference

A statistically significant correlation in the intensity of pain measured by the VAS scale and DA was observed. The correlation coefficient values obtained before and after therapy indicate the existence of a statistically significant association, but the absolute values of the coefficients in both cases were less than 0.5, indicating the existence of significant deviations between the methods – that is, great subjective pain influence and evaluation on the VAS scale in respondents (Table 4).

Table 4. Correlation between different methods of measuring intensity of pain

Correlation	VAS′	VAS″	p
DA′	R = -0.473		0.001*
DA″		R = -0.472	0.001*

DA′ – digital algometer before the therapy; DA″ – digital algometer after the therapy; VAS′ – visual analogue scale before the therapy;
VAS″ – visual analogue scale after the therapy;
*statistically significant correlation

By a correlation analysis of the current intensity value of the pain shown by the numerical scale and the score of pain in the VAS scale, a statistically significant correlation was noted in the assessment of the pain measured by these instruments. Despite similar criteria of pain assessment with these methods, the absolute value of the coefficient of correlation points to significant deviations in the assessment of the respondents for the same pain intensity experience (Table 5).

Table 5. Correlation of pain levels assessed in different ways

Correlation	VAS′	DA′	p
NS	R = 0.510		0.000*
		R = -0.293	0.053

VAS′ – visual analogue scale before the therapy; DA′ – digital algometer before the therapy; NS – current pain;

*statistically significant correlation

In order to evaluate the efficiency of different therapeutic modalities for pain reduction, a multivariate regression model was used, where the severity of pain after treatment was assessed by the VAS and DA methods. In this regression model, the effect of all observed risk factors, pretreatment factors, applied therapies, and other outcomes (depression, psychosocial status) were evaluated, on the evaluation of pain by the VAS and DA methods after therapy.

Table 6. Uni- and multivariate regression analysis related to VAS″

Observed risk parameters	Univariate		Multivariate $R^2 = 0.528$	
	#B (95%CI)	p	B (95%CI)	p
Sex	6.529 (-8.326–21.384)	0.380	/	/
Age	0.179 (-0.327–0.686)	0.479	/	/
VAS′	0.608 (0.378–0.838)	0.000*	0.426 (0.115–0.737)	0.009*
DA′	-2.658 (-4.418–-0.899)	0.004*	-0.931 (-2.655–0.794)	0.281
Therapy	1.315 (-6.336–8.965)	0.731	/	/
Diagnosis	-1.546 (-8.974–5.881)	0.677	/	/
Working ability	3.211 (0.818–5.604)	0.010*	-3.024 (-7.327–1.279)	0.162
Social life	4.088 (1.732–6.444)	0.001*	4.517 (0.516–8.517)	0.028*
Everyday activity	2.600 (0.287–4.912)	0.029*	0.186 (-2.314–2.687)	0.881
Level of chronic pain	12.643 (1.944–23.342)	0.022*	-2.776 (-13.366–7.814)	0.598
Reduction of function	5.182 (1.159–9.205)	0.013*	3.334 (-0.231–6.900)	0.066
Depression	9.082 (1.696–16.467)	0.017*	1.164 (-6.141–8.468)	0.748

VAS″ – visual analogue scale after the therapy; VAS′ – visual analogue scale before the therapy; DA′ – digital algometer before the therapy;
*statistically significant;
#non-standardized coefficient B

In the measurement of VAS pain by scaling, a univariate regression analysis found that pain after the applied therapy was associated with the pain described before the start of treatment, the assessment of working ability, social life, everyday activities, chronic pain, reduction of orofacial functions, and depression (Table 6). The intensity of pain measured prior to the therapy by the VAS and the assessment of social life were singled out, as the predictors

of post-therapeutic intensity of pain. Respondents who complained of severe pain before initiating therapy had a higher intensity of pain after the applied treatment. In all subjects with pain in the orofacial region, regardless of pain reduction after therapy, one can always expect the influence of pain on their social life, which is more disturbed as the pain is stronger.

When assessing post-treatment pain measured with the DA, the univariate regression analysis as statistically significant included sex, strength of the pain measured by the DA before treatment, and the pain level after treatment measured on the VAS scale. Multivariant regression analysis, the severity of pain measured by the DA before therapy and the measurement of VAS after therapy, have been singled out as factors with an independent impact on the severity of pain, measured by the same method after therapy (Table 7).

Table 7. Uni- and multivariate regression analysis related to digital algorithm measurement (DA")

Observed risk parameters	Univariate		Multivariate R2	
	#B (95%CI)	p	B (95% CI)	p
Sex	-2.868 (-5.294–-0.442)	0.022*	-0.690 (-2.638–1.258)	0.478
Age	-0.024 (-0.112–0.063)	0.577	/	/
VAS'	-0.040 (-0.090–0.010)	0.115	/	/
DA'	0.766 (0.530–1.001)	0.000*	0.634 (0.358–0.911)	0.000*
Therapy	-0.022 (-1.343–1.299)	0.973	/	/
VAS"	-0.081 (-0.129–0.034)	0.001*	-0.036 (-0.129–0.034)	0.005*
Diagnosis	-0.430 (-1.706–0.847)	0.501	/	/
Working ability	0.023 (-0.424–0.470)	0.919	/	/
Social life	-0.257 (-0.712–0.197)	0.260	/	/
Everyday activity	0.147 (-0.273–0.567)	0.483	/	/
Level of chronic pain	-1.337 (-3.258–0.584)	0.167	/	/
Reduction of function	-0.423 (-1.158–0.313)	0.253	/	/
Depression	-0.323 (-1.683–1.036)	0.634	/	/

DA' – digital algometer before the therapy; DA" – digital algometer after the therapy; VAS' – visual analogue scale before the therapy; VAS" – visual analogue scale after the therapy;
*statistically significant;
#non-standardized coefficient B

DISCUSSION

Pain is not only a signal of tissue damage, but also a difficulty in most dental procedures, delaying the rehabilitation of functions and reducing the chances of a patient returning. Pain control is often inadequate, either due to insufficient analgesia or due to unacceptable side effects of drug therapy. In addition, inadequate analgesia can contribute to the onset of hyperalgesia during the recovery period. The aforementioned facts indicate that it is imperative to have effective analgesia with minimal side effects. Pain, as a symptom of TMDs and associated dysfunction of the mastication muscles and TM joints, is a significant entity of TMD. A simple and reliable determination of the origin of pain is detrimental for the choice of therapeutic modality. Multifactorial etiology and overlapping of symptoms and signs of various TMDs complicate this requirement [7]. An additional problem in the choice of therapeutic approach lies in the fact that pain, as the most prominent symptom, can occur secondary, as a result of disorders of adjacent structures. Since the causes of TMD and the interaction between different entities of TMD are very complex, initial therapy should be non-invasive and reversible. In this respect, occlusal splint represents the therapy of choice, since it temporarily improves the functional relationship of the structures of the orofacial (OF) system. The occlusal splint, acting on the cause of the disorder, influences symptoms, and also plays a role as a diagnostic agent. This fact is particularly important in cases when there is a suspicion of the dominant influence of occlusal factors in the development of TMD. Detailed mechanisms by which occlusal splints achieve these results are still the subject of discussion [8]. Stabilization splint is sometimes referred to as the relaxation splint due to its primary application in the reduction of muscle pain [9].

The results of this study indicate a positive effect of the stabilization splint in the reduction of painful symptoms regardless of the TMD, as there is a statistically significant difference in the measured intensity of pain in all treatment groups before and after the applied therapy ($p \leq 0.05$). All subjects of the clinical population who were male (15.9%) were treated physically with an occlusal stabilization splint exclusively. In the therapeutic group treated with a stabilization splint (55%), the majority of subjects had a diagnosed musculoskeletal dysfunction. The majority of respondents with moderate depression were in the treatment group treated with occlusal splint (45%), as well as subjects without defined depression (45%). Positive effects of stabilization pain therapy in pain reduction were observed in many studies [10–14]. Stabilization splints, as splints of flat surfaces, are conventionally made of solid material. Such splints are resistant to the long-lasting effect of occlusal forces of varying intensity and satisfies the requirements of physiologically optimal and stable occlusion [15]. Solid-type splints reduce the electromyographic activity of the masseter and temporal muscles [16].

Lazić et al. [17] carried out a comparative analysis of the mechanical and chemical properties, structure, surface of PMMA breaks, and thermoplastic polymers. The results of the tests indicate that thermoplastic polycarbonate (TPK) materials are more suitable for making occlusal splints, since the beginning of the deformation is elastic, and they also have a potency of flow and characteristics of viscoelastic polymers. Mechanical properties and appearance of faulty surfaces imposes the use of TPK materials for making occlusal splints [17].

The choice of splint as a therapeutic agent in the treatment of painful TMDs requires caution and a properly diagnosed dysfunction. Also, limited therapeutic capacity should be taken into account as well as possible complications during the wearing of such compensation (caries of the tooth below the splint, gingivitis due to poor oral hygiene, difficult speech and breathing functions, and eventual psychosomatic reactions to foreign bodies). These facts imply the obligation to conduct regular and frequent check-ups after giving splint to the patient.

Given that the studied population consisted of patients who sought help regarding treatment of TMD, we can say that respondents belong to the clinical population. Of the 44 patients in the clinical population who exposed the signs and symptoms of TMD, 22 subjects (50%) had a combined musculoskeletal dysfunction. Fifteen respondents (34.1%) showed symptoms of articular dysfunction regardless of the possibility of condyle reduction or of the degree of mouth opening, and seven respondents (15.1%) showed symptoms and signs of muscular dysfunction regardless of the degree of mouth opening. In this regard, the results of the study on the distribution of various subgroups of TMD are similar to the results of many studies [18, 19, 20].

Differences in the frequency and distribution of TMD subgroups are due to different criteria of homogenization of the examined population and various diagnostic methods. In addition, there is a difference in the type of population surveyed (clinical or general), as well as in the age of the population group.

By analyzing the distribution of TMD among the sexes in the clinical population, the results show that the incidence of symptoms and signs of TMD is six times higher in females than in males. Of the 44 subjects who were included in the study, 15.9% of the respondents were male. The high incidence of TMD in women is considered to be the consequence of greater responsibility of women towards their own health and more frequent visits to the doctor, and that women are more affected by stress [20, 21].

The available methods vary significantly among researchers, which does not allow for a comparison of different studies. The most common problems in comparing the results of other studies lie in different time frames given to respondents to evaluate the pain. While some researchers require information on the current intensity of pain, others require that respondents rank the pain level over the previous 24 hours. This is one of the reasons for the existence of variability of the results [22, 23].

A statistically significant correlation was observed in the measured intensity of pain with the VAS scale and the DA method, which also indicates the existence of significant discrepancies between the measurement methods, i.e. the great influence of subjective pain experience and the assessment on the VAS scale. In addition, some studies point to the unreliability of the digital algorithm method by pressing force in successive measurements [24].

The inconsistency of the results of the multivariate regression analysis for pain measured by the VAS scale and the algometric method after the applied treatment is another confirmation of the quality of VAS as an instrument for subjective assessment of the pain experience. Given that the experience of pain is an individual category involved in the psychosocial life of an individual, despite the bias that the VAS scale implies in the assessment of pain, a comparative application of the VAS scale with other instruments for measuring the intensity of pain is necessary.

In any case, one should be cautious in interpreting the results for at least three reasons. The first one is that patients with chronic pain have normal adjustment to the existing conditions, whether or not therapy is performed, and symptom regression occurs. Another reason for symptom regression is the consequence of the doctor–patient interaction. Patient encouragement and information on the causative agent and benign character of the disease leave a positive effect on the patient and his presentation of the symptoms of pain [25].

The third reason lies in the fact that the pain regression is also influenced by psychosocial factors, primarily the quality of life, social and cultural status, and previous painful experiences [26].

CONCLUISION

The study found that the intensity of the pain is not a predictor of the dysfunction of the orofacial system. Considering the objectives of the study, the analysis of the obtained results suggests that therapy with an occlusal stabilization splint can significantly reduce the pain intensity and confirmes the positive analgesic effect of occlusal stabilization splint in TMD patients. All therapeutic modalities applied in this study have proved to be equally effective in reducing painful symptoms so that the prognostic significance of the intensity of pain measured before treatment is irrelevant. Significant deviation in respondents' assessments of the same pain intensity experience, depending on the type of the measuring instrument, was also found.

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Conflict of interest: None declared.

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Оклузални сплинт – алтернатива у терапији болесника са темпоромандибуларним дисфункцијама

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САЖЕТАК

Увод/Циљ Бол порекла мишићно-скелетних структура мастикаторног система представља један од симптома који припадају категорији темпоромандибуларних поремећаја или темпоромандибуларних дисфункција (ТМД).

Циљ истраживања је био да се процени ефекат терапије стабилизационим оклузалним сплинтом у контроли болних симптома ТМД у поређењу са ефектом терапије лековима.

Методе Коришћењем стандардног дијагностичког протокола (*RDC*/ТМД) предложеног од стране Дворкина и Лерешеа, издвојена је група од 44 болесника са болним темпоромандибуларним дисфункцијама. Болесници су подељени у три терапијске групе случајним избором. Прва група је подвргнута терапији стабилизационим оклузалним сплинтом у периоду од месец дана. У две контролне групе је спроведена терапија нестероидним антиинфламаторним леком ибупрофеном (бруфен, *Mylan*) или комбинацијом ибупрофена и лека из групе бензодиазепина – диазепама (диазепам, Хемофарм) у периоду од три недеље. У циљу процене ефеката терапије стабилизационим оклузалним сплинтом и терапије лековима, пре и после спроведене терапије изведена су мерења интензитета бола визуелном аналогном скалом и дигиталним притисним алгометром.

Резултати У све три терапијске групе постигнуто је значајно смањење интензитета болних симптома. Нису забележене значајне разлике у успешности смањења бола између предложених терапијских модалитета.

Закључак Добијени резултати потврђују да је терапија стабилизационим оклузалним сплинтом валидна процедура у смањењу бола код болесника са ТМД.

Кључне речи: темпоромандибуларне дисфункције; оклузални сплинт; фармакотерапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Tissue plasminogen activator for dysfunctional tunneled vascular catheters for hemodialysis – single center experience

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SUMMARY

Introduction/Objective Thrombosis of hemodialysis catheters is one of the major complications, which leads to catheter dysfunction. Although tissue plasminogen activator has been proven to be effective in reestablishing blood flow rate through dysfunctional catheters, clinical data in Serbia are missing. The objective of the study was to analyze tissue plasminogen activator efficacy in reestablishing blood flow rate and the influence on catheter survival.

Methods The study included 53 tunneled catheters from 32 patients on hemodialysis. After catheter dysfunction was established, 580,000 units of tissue plasminogen activator was applied into each catheter lumen for about two hours before hemodialysis. The criteria for success was blood flow rate on the next hemodialysis – over 200 mL/minute was considered to be complete success, 180–200 mL/minute partial success, and under 180 mL/minute was considered a failure.

Results Out of 53, 25 catheters (47%) had dysfunction with an incidence of 3.8/1,000 catheter days. Catheters placed in femoral veins, "after-first" catheters, catheters with infection, and catheters in older patients had higher risk for dysfunction. Multivariate logistic regression analysis confirmed that only older age was significantly related to catheter dysfunction. Of the total of 50 applications of tissue plasminogen activator, 35 (70%) were successful, seven procedures (14%) were partially successful and eight (16%) dysfunctional catheters failed to respond to therapy. Six-, 12- and 24-month survival was 87%, 81%, and 20%, respectively, for catheters without dysfunction, and 71%, 47.5%, and 12%, respectively, for catheters with dysfunction.

Conclusion Tissue plasminogen activator dosing is noninvasive, efficient, and safe in reestablishing blood flow rate through dysfunctional catheters, thus prolonging catheters life and sparing patients from additional vascular procedures.

Keywords: hemodialysis; tunneled vascular catheters; catheter thrombosis; tissue plasminogen activator

INTRODUCTION

Adequate vascular access is crucial for successful hemodialysis (HD) treatment. Ideal access provides adequate blood flow rate (BFR) during an HD session and an adequate dialysis dose. Also, it has a few complications in the long term [1]. According to Vascular Access Society guidelines, arterial-venous fistula (AVF) is considered the best vascular access, based on its longevity and the rarity of complications [2]. If blood vessels were inadequate for the creation of AVF, then the creation of arterial-venous graft (AVG) should be considered. AVG provides adequate BFR during an HD session, but complications such as infection and thrombosis are more frequent comparing to AVF [1].

Tunneled vascular catheters (TVC) are used in patients whose blood vessels are exhausted for the creation of AVF or AVG, in patients with severe peripheral vascular disease, and in those with short life expectancy [3]. Although they are ready for use right after the insertion, providing satisfactory BFR, the rate of complications is discouraging. In regard to complications, femoral veins are considered to be the worst position for catheter placement, especially compared to the right internal jugular vein, which is usually recommended [4]. According to studies, six-month survival of TVC is 60%, and one-year survival is 40% [5].

National Kidney Foundation Dialysis Outcomes and Quality Initiative defined catheter dysfunction as a failure to attain and maintain an extracorporeal BFR of 300 mL/minute or greater at a prepump arterial pressure lower than -250 mmHg, and increased venous resistance (> 250 mmHg) [1]. Catheter dysfunction is also considered if Kt/V is lower than 1.2, or if urea reduction rate (URR) is < 65%, without extending HD.

The most common complications related to the catheters are infection and thrombosis [6]. They usually require catheter replacement, which is an invasive procedure accompanied by many complications: pneumothorax (0.2% if the internal jugular vein is punctured, and 3.1% if the subclavian vein is punctured), artery puncture (9.4% in attempt to puncture the internal jugular vein, 4.9% for the subclavian

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vein, and 15% for the femoral vein), bleeding (3%), hemothorax (0.6%), arrhythmias (0.9%), malposition (1%), perforation of the right atrium [7, 8].

Risk factors for thrombosis may be related to the catheter or to the patient. Catheter duration and catheter lumen width are directly proportional to thrombosis rate [9]. Risk factors related to patients are heart failure, infections, and malignant tumors [10]. In order to prevent catheter thrombosis, anticoagulants (heparin or sodium citrate) are placed in catheter lumens between two HD sessions. Theoretically, tissue plasminogen activator (TPA) can be used for the prevention of catheter thrombosis, but it is still debatable considering the cost–benefit relation. Therefore, it is mostly used for the treatment of acute catheter thrombosis [11, 12]. TPA translates plasminogen to plasmin, which is a powerful proteolytic enzyme that degrades fibrin fibers and other coagulation proteins [13].

According to some literature data, local application of TPA in catheter lumens is safe and efficient [14]. In clinical practice, there are no guidelines for the optimal dose of TPA. In a retrospective cohort study, Yaseen et al. [15] compared efficacy of 1 mg of TPA and 2 mg of TPA per catheter lumen and results revealed that catheter survival was better after using 2 mg of TPA per catheter lumen. Also, it has been shown that the risk for catheter replacement due to non-resolved obstruction is 2.75 times greater after using 1 mg of TPA per catheter lumen. Macrae et al. [16] compared one hour to 48 hours TPA dwell, and there was no statistically significant difference between the short and long TPA dwell groups for catheter patency at the subsequent HD run (76.9% *vs*. 79.4%) or at two weeks (42.3% *vs*. 52.9%).

The objective of this prospective study was to analyze the efficacy and safety of TPA application on reestablishing blood flow through dysfunctional TVC, and to confirm the influence of TPA on catheter survival.

METHODS

Patients and catheters

This prospective study examined all TVC (Hickman, Bard, Salt Lake City, UT) placed between March 1, 2012 and December 1, 2014 in patients treated with chronic HD in Clinical Department for Renal Diseases, Zvezdara University Medical Center, Belgrade.

A database was constructed based on the patient's medical documentation. All patients were dialyzed three times weekly, for four hours. Patients were followed up from the day of catheter insertion to the day of catheter removal, death, or the end of the study period. The catheters were inserted by vascular surgeon under local anesthesia, without radioscopic or ultrasound guidance. After catheter placement, X-ray was performed to ensure adequate catheter position. Only catheters that were functional at least three consecutive HD after insertion were analyzed.

Some patients had more than one catheter, since catheters were replaced due to the complications. Second and following catheters were simply called "after-first" catheters. Most of the patients had AVF and/or AVG and/or peritoneal dialysis (PD) before catheter placement.

According to the unit protocol, catheter dysfunction was defined as the difficulty in infusing or withdrawing blood from their lumens. Risk factors for catheter dysfunction were evaluated, including sex, age, length of dialysis, comorbidities (hypertension and diabetes mellitus), associated infections, use of antiplatelet and oral anticoagulant therapy (OACT), laboratory analyses (albumin and hemoglobin level), and catheter location.

TPA application

Due to acute catheter dysfunction, patients received 580,000 units (1 mg) of TPA into each catheter lumen. TPA was diluted with saline in final concentration that fits every catheter lumen. Dwell time was two hours before HD.

The criterion for success was BFR on subsequent HD session was as follows: over 200 mL/minute was considered to be complete success, 180–200 mL/minute partial success, and under 180 mL/minute was considered failure of therapy. Criteria for catheter function/dysfunction are not clearly stated by current guidelines or literature data, since dialysis adequacy is the main criteria for catheter replacement. Therefore, decision about catheter dysfunction and removal is usually brought according to BFR, dialysis adequacy and patient's residual renal function. Still, it is desirable to achieve BFR of more than 200 mL/minute for adequate HD. If BFR is less than 200 mL/minute (180–200 mL/minute), adequate dialysis could still be achieved by selection of dialyzer of higher surface area and by prolonging dialysis time, particularly if the patient has preserved residual renal function. Therefore, we designated such flow rate as partial success and it was still functional catheter, with no need for removal. However, with BFR less than 180 mL/minute, adequate HD can hardly be achieved and therefore we assumed these catheters failed (dysfunctional).

Since catheter thrombosis is the most common cause of catheter dysfunction, we performed X-ray diagnostic procedures to determine the etiology of dysfunction only if the second dose of TPA failed to provide BFR through catheter.

Statistical analysis

SPSS Version 15.0 (SPSS Inc. Chicago, IL, USA) was used to analyze the data. Descriptive analysis was applied to study the characteristics of the study population and of the catheters. Student's t-test was performed for intergroup comparison for variables with normal distribution. For variables without normal distribution, differences between the groups was analyzed with the Mann–Whitney test. Kaplan–Meier curves were constructed for catheter survival. We censored for events that led to catheter removal such as catheter bacteremia, the transition to an AVF or the start of PD, and patient death. Logistic regression analysis was applied to study the influence of covariates on the incidence of catheter dysfunction. Independent variables were age, sex, comorbidities (hypertension and diabetes mellitus), associated infections, OACT, antiplatelet therapy, hemoglobin and albumin level,

length of dialysis, and catheter location. The dependent variable was catheter dysfunction (0 for catheters without dysfunction and 1 for catheters with dysfunction). Statistical significance for all comparisons was set at $p \leq 0.05$.

RESULTS

Patient characteristics

The median length of the follow-up was seven months (range 1–32 months). Study included 16 men (50%) and 16 women (50%) of the average age of 62 ± 14 years (Table 1). Most of the patients had hypertension (81%), and 22% of them had diabetes mellitus. Only five patients (16%) started dialysis with TVC, while the others were on dialysis for 38 ± 52 months before they had their tunneled catheters placed. Most of the patients used AVF before catheter (78%), 37.5% had AVG, and 31% were treated with PD.

Table 1. Data on the patients and catheters

Parameter	Patients, n = 32
Male sex, n (%)	16 (50)
Age (X ± SD)	62 ± 14 (min. 30, max. 80)
Diabetes mellitus, n (%)	7 (22)
Hypertension, n (%)	26 (81)
Dialysis duration before catheter (months), (X ± SD)	38 ± 52 (min. 1, max. 196)
Previous access for dialysis: – patients with AVF, n (%) – patients with AVG, n (%) – switch from PD, n (%) – catheter as the first access, n (%)	 25 (78) 12 (37.5) 10 (31) 5 (16)
Hemoglobin concentration (≥ 95 g/L), n (%)	22 (69)
Albumin concentration (< 35 g/L), n (%)	9 (28)
Usage of antiplatelet therapy, n (%)	16 (50)
Usage of OACT, n (%)	4 (12.5)
Overall number of catheters	53
Number of dysfunctional catheters, n (%)	25 (47)
Time to first dysfunction (days), median (IQR) – first catheters – after-first catheters	 235 (304) 100 (151)
Incidence of dysfunction per 1,000 catheter days	3.8/1,000 catheter days
TPA application per dysfunctional catheter (X ± SD)	2 ± 1.8 (min. 1, max. 9)
Number of catheter-related bacteremia, n (%)	16 (30)

AVF – arterial-venous fistula; AVG – arterial-venous graft; PD – peritoneal dialysis; OACT – oral anticoagulant therapy; TPA – tissue plasminogen activator; IQR – interquartile range

Twenty-two patients (69%) had a desirable hemoglobin level, but almost one third of them had albumin concentration below the lower limit. Half of them were using antiplatelet therapy, and 12.5% were using OACT.

During the study, 53 catheters were placed in 32 patients. The maximal number of catheters received by a single patient over the study period was four. Out of 53 catheters, 25 (47%) had dysfunction which required the use of TPA once or repeatedly. Incidence of dysfunction was 3.8/1,000 catheter days.

Time to the first catheter dysfunction varied 6–670 days (median being 110 days). We investigated if there was difference in time to first dysfunction between the first catheters and the after-first catheters. Since data were nonparametric, we performed the Mann–Whitney test and confirmed that there was statistically significant difference between the first and the after-first catheters regarding the time to the first dysfunction ($p = 0.043$).

The number of TPA applications per dysfunctional catheter was 2 ± 1.8 (min. 1, max. 9). Sixteen catheters (30%) had catheter-related bacteremia, but there was no significant difference in catheter dysfunction between catheters with or without infection ($p = 0.14$).

Table 2 presents the success of the TPA procedure. In 25 dysfunctional catheters, 50 TPA applications were performed. In 35 applications (70%), the use of TPA was followed by adequate HD session. In seven applications (14%), partial success was achieved, and eight (16%) dysfunctional catheters failed to respond to therapy with TPA. We didn't find any statistically significant difference in success between the first, the second, and subsequent TPA application per catheter ($p = 0.9$).

Table 2. Number of successful tissue plasminogen activator procedures (success, partial success and failure)

Number of TPA procedures per catheter	Immediate success of TPA procedure		
	Success (BFR > 200 mL/minute) n = 35	Partial success (BFR 180–200 mL/minute) n = 7	Failure (BFR < 180 mL/minute) n = 8
First	18 (72%)	3 (12%)	4 (16%)
Second	8 (66.7%)	2 (16.7%)	2 (16.7%)
Subsequent (3–9)	9 (69.2%)	2 (15.4%)	2 (15.4%)

BFR – blood flow rate; TPA – tissue plasminogen activator

Also, there was no difference in success rate of the TPA procedure between the first and the after-first catheters ($p = 0.57$).

Catheter survival

As a prediction, if catheters had been removed after the first dysfunction without TPA therapy, one-year survival of dysfunctional catheters would have been only 12% (Figure 1). Figure 2 shows survival curves for the catheters with and without dysfunction and TPA intervention. Six-, 12- and 24-month survival was 87%, 81%, and 20%, respectively, for catheters without dysfunction, and 71%, 47.5%, and 12%, respectively, for catheters with dysfunction in which TPA therapy was applied. Log rank test was performed and a statistical difference between two Kaplan–Meier curves was not confirmed ($p = 0.1$).

In nine dysfunctional catheters (36%) after one use of TPA, catheters continued to function without any need for additional TPA procedures. In three catheters (12%) after the second unsuccessful dose of TPA, diagnostic procedures were performed and an X-ray revealed secondary catheter malposition, which required catheter replacement.

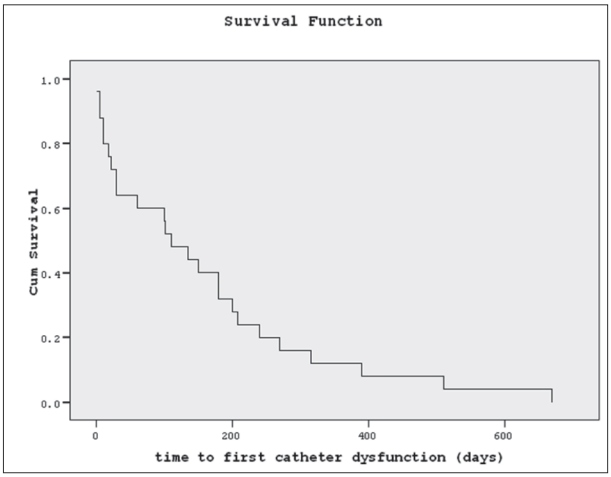

Figure 1. Prediction of overall catheter survival without tissue plasminogen activator procedure (Kaplan–Meier survival curve)

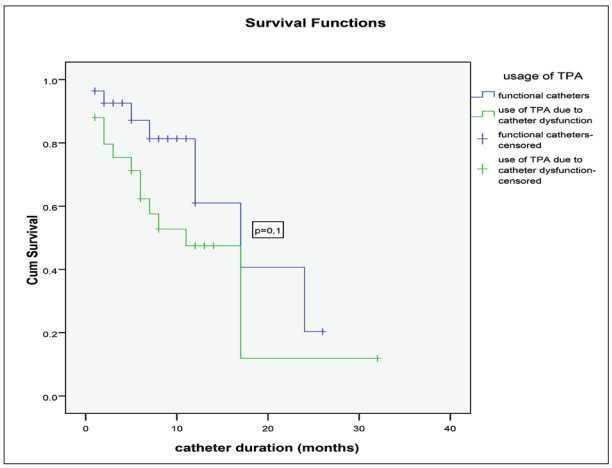

Figure 2. Kaplan–Meier survival analysis for functional and dysfunctional catheters treated with tissue plasminogen activator

Risk factors for catheter dysfunction

Table 3 shows the results of multivariate logistics regression analysis. Only older age was significantly related to catheter dysfunction, but not the use of antiplatelet and OAC drugs, sex, comorbidities (hypertension, diabetes mellitus), laboratory analyses (albumin and hemoglobin level), and the length of HD. Femoral location of the catheter had three times higher risk for developing dysfunction compared to jugular and subclavian localization, but this difference did not reach statistical significance. Concomitant bacteremia increases the risk for dysfunction two-fold, and the after-first catheters are at 1.5 higher risk compared to the first catheters, both without statistical significance.

Table 3. Variables associated with dysfunction of tunneled vascular catheters

Covariates	B	Exp (B)	p	95% CI
Age	0.045	1.046	0.036	0.003–1.091
Association with infection	0.752	2.122	0.269	0.559–8.058
Femoral veins	1.093	2.982	0.164	0.640–13.892
After-first catheters	0.407	1.503	0.548	0.398–5.665

DISCUSSION

This study confirmed that 25 (47%) out of 53 examined catheters had dysfunction that required the use of TPA, with the incidence of dysfunction of 3.8/1,000 catheter days. The literature data revealed lower incidence; Develter et al. [17] described 1.94 dysfunctions per 1,000 catheter days, while Lee et al. [18] found three dysfunctions per 1,000 catheter days, and the difference could be explained by different patient populations – our patients were older and had higher comorbidity, including diabetes mellitus.

By using logistic regression analysis, we confirmed that age was the only significant risk factor for catheter dysfunction. Timsit et al. [19] also showed that older patients are at a higher risk for developing catheter thrombosis. This might be due to many comorbidities and damaged blood vessels that are more frequent in the elderly, which makes them prone to thrombosis.

The relationship between catheter-related bacteremia and thrombosis has been proven by literature data [19, 20]. It is still debatable whether infection promotes thrombosis or vice versa. One of the possible explanations could be that the fibrin sheath surrounding catheters increases bacterial adherence [21]. Vaudaux et al. [10] in their study suggested that host factors, such as fibrin, fibronectin, and fibrinogen, may have a significant role in staphylococcal adherence, colonization, and infection by interacting with intravascular catheters. According to our data, only 30% of dysfunctional catheters were associated with bacteremia. Therefore, it is difficult to confirm the clear relationship between two events.

As shown in previous studies, femoral approach is associated with higher risk of thrombotic complications, which is also proved by our study, although without statistical significance. The most striking results were shown by Merrer et al. [22], who compared subclavian and femoral approach, where femoral approach proved to be the most unfavorable one (1.9% *vs.* 21%).

A systematic review was performed to evaluate studies that examined the efficacy and safety of thrombolytic therapy in dysfunctional HD catheters [23]. The success rate was higher with reteplase (88%), followed by TPA (81%), and tenecteplase (41%).

In our study, the use of TPA proved to be successful in re-establishing BFR in subsequent HD in 70% of the procedures. These results are in compliance with the study by Ponce et al. [24], according to which adequate BFR on the following HD session was achieved in 77% of dysfunctional catheters after one TPA dose, in 10% after the second dose, and only 13% of catheters failed to respond to treatment. On the other hand, Little and Walshe [25] showed that the cumulative gain of repeated use of TPA in an attempt at thrombolysis is small. Authors stated that if the TPA is required more than once, it might be that the catheter has been structurally altered.

Since 1993, the use of TVC for HD has increased from less than 10% to more than 30%, as revealed by the US Renal Data System [26]. Data for Serbia in 2012 have shown that 89% of the prevalent patients used AVF as the

vascular access for HD and 3.1% used AVG. The percentage of prevalent patients with TVC was 3.5%. During 2012, 88% of the patients started HD with AVF, 4% with AVG, and 7.8% with TVC, thus showing a growing trend [27].

In our study, one-year survival of dysfunctional catheters treated with TPA was 47.5%. As a prediction, if catheters had been replaced after the first dysfunction, one-year survival would have been only 12%, as revealed by the Kaplan–Meier analysis. Log rank test did not confirm any statistically significant difference in survival between functional and dysfunctional catheters, in which TPA therapy was applied. This finding proves that TPA is successful in prolonging dysfunctional catheters life and saving patients from additional interventions, since the most of them have no alternative to another vascular approach.

In our study, there were no adverse effects of the TPA therapy. Previously mentioned systematic review also reported extremely rare adverse effects of thrombolytic therapy, most likely due to limited systemic exposure to TPA [23].

There are some limitations to our study. In addition to the small study population and the number of catheters, etiology of catheter dysfunction was examined after failure of the second dose of TPA, so secondary malposition was overlooked in three catheters. Therapeutic success of TPA was evaluated by the BFR, but not with color Doppler imaging and dialysis adequacy (Kt/V) which could be a useful diagnostic tool in case of recirculation over the catheter.

CONCLUSION

This prospective single-center study provides data on permanent tunneled vascular catheters for HD with an acceptable dysfunction rate (3.8/1,000 catheter days). If dysfunction occurs, TPA is proven to be efficient, safe, easy to perform, and without significant disruption to the dialysis schedule. It has also shown that TPA extends catheter longevity in patients with exhausted other alternatives for dialysis.

Conflict of interest: None declared.

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Ткивни активатор плазминогена код дисфункционалних тунелизованих васкуларних катетера за хемодијализу – искуство једног центра

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САЖЕТАК

Увод/Циљ Тромбоза катетера за хемодијализу је једна од најчешћих компликација која доводи до дисфункције катетера. Ткивни активатор плазминогена се показао ефикасним у решавању ове компликације, али клинички подаци у Србији недостају.

Циљ рада је испитивање ефикасности ткивног активатора плазминогена у поновном успостављању протока крви преко дисфункционалног катетера и утицаја на век трајања катетера.

Методе У студију је укључено 53 тунелизованих катетера код 32 болесника на хемодијализи. По утврђивању дисфункције, примењено је по 580.000 јединица ткивног активатора плазминогена у сваки лумен катетера два сата пре хемодијализе. Критеријум за терапијски успех је био проток крви на наредној хемодијализи: преко 200 *ml/min.* се сматрало комплетним успехом, од 180–200 *ml/min.* делимичним успехом и испод 180 *ml/min.* неуспехом.

Резултати Од 53 испитивана катетера, 25 (47%) њих је имало дисфункцију са учесталошћу 3,8/1000 катетер дана. Већи ризик за дисфункцију су имали катетери пласирани у феморалним венама, „наредни" катетери, катетери са придруженом инфекцијом и катетери код старијих болесника. Мултиваријантна регресиона анализа је потврдила да катетери код старијих болесника имају статистички значајно већи ризик за дисфункцију. Од укупно 50 примена ткивног активатора плазминогена било је 35 (70%) успешних, седам (14%) делимично успешних и осам (16%) неуспешних покушаја остваривања адекватног протока крви преко дисфункционалног катетера. У групи катетера који нису имали дисфункцију, након шест, 12 и 24 месеца проценат функционалних катетера је износио 87%, 81% и 20%, док је у групи катетера са дисфункцијом тај проценат био 71%, 47,5% и 12%.

Закључак Примена ткивног активатора плазминогена је неинвазивна, ефикасна и безбедна за поновно успостављање протока крви преко дисфункционалног катетера, уз продужетак века трајања катетера и поштеду болесника од додатних васкуларних интервенција.

Кључне речи: хемодијализа; тунелизовани васкуларни катетери; тромбоза катетера; ткивни активатор плазминогена

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The influence of the expression of steroid receptors on angiogenesis, proliferation and apoptosis in myomas of pre- and postmenopausal women

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SUMMARY

Introduction/Objective The aim of this study was to determine the effects of the estrogen and progesterone receptor status on angiogenesis, proliferation, and apoptosis of myoma cells in premenopausal (PreM) and postmenopausal (PostM) women.

Methods This was a cross section; clinical-experimental, retrospective, non-interventional study in the field of the study of fundamental pathogenesis mechanisms of disease using pathohistological materials from the existing archive. The research included 76 patients diagnosed with uterine leiomyomas, operatively treated in the Clinic for Gynecology and Obstetrics, Clinical Centre Kragujevac, Serbia. According to the menstrual status, we formed two experimental subgroups. The first group was PreM women (n = 35; 46.2 ± 5.02 years old), and the second group was PostM women (n = 41; 60.25 ± 5.41 years old). Hematoxylin-eosin staining for myoma and myometrium was conducted, as well as immunohistochemistry for ERα, ERβ, PRa, vascular endothelial growth factor, endoglin, Ki67, and caspase-3.

Results Progesterone receptor was overexpressed in myoma and myometrium of PreM compared to myoma and myometrium of PostM women. Expression of caspase-3 was a statistically significant increase in PostM women compared to PreM group. ERα and ERβ were not changed among groups neither in myoma nor in myometrium samples.

Conclusion According to our data, PRa had higher influence on apoptosis and cell growth than estrogen receptors. Since PRa was increased in PreM in both myoma and myometrium, probably this expression led further to lower expression of apoptotic marker in PreM women.

Keywords: steroid receptors; apoptosis; angiogenesis; premenopausal; postmenopausal

INTRODUCTION

Uterine fibroids, also known as uterine leiomyomas or fibroids are well-limited, pseudoencapsulated, benign, monoclonal tumors, composed mainly of smooth muscle cells of the uterus uterine leiomyomas, are among the most frequent gynecological tumors in the reproductive period of women. Independently or in association with hyperplasia and adenomyosis, they reach an incidence of 77%, often causing clinically complicated bleeding, which is why they are the leading cause for hysterectomy and a major global health problem [1, 2]. It has been known that uterine leiomyomas are a hormone-dependent disease. However, the mechanism of action is still unknown, and there is increasing evidence that steroid hormones, estrogen and progesterone receptors are not the only modulators of myoma growth [2, 3, 4]. This can be explained with a presence of similar level of circulating hormone in women with and without myoma, with the occurrence of hormone-independent extrauterine leiomyoma and the possible absence of their regression in postmenopausal women [5].

Throughout the last decade, high effort has been invested to clarify the role of gonadal steroids, the expression of local growth factors, and factors associated with apoptosis in myoma cells. Recent studies showed local tissue-specific factors (for example, growth factors), as well as somatic mutations in pro and antiapoptotic genomes, participate in the pathogenesis and progression of these tumors, with or without cross-linking with mechanisms of action of steroid hormones. Among the environmental factors, particular attention is drawn to the presence and effect of estrogen and progesterone receptors on the endometrium and myometrial cells in the uterine wall with myoma [3].

The key pathological processes involved in myoma growth are proliferation and hypertrophy of leiomyocytes, apoptosis, angiogenesis, stromal and secondary changes [6]. The most reliable marker of cell proliferation is Ki-67 or a proliferation-cell nuclear antigen, which denotes not only the cells in the divide, but also

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all those in the synthetic phase of the cell cycle [7]. A high level of Ki-67 antigen, detected during the secretory phase, suggests that progesterone has a synergistic effect in the pathogenesis of myoma [8].

Apoptosis is a process of programmed cell death that eliminates dysfunctional and undesirable cells. It is highly regulated by the complex interaction between the pro- and the anti-apoptotic molecules, is performed in one cell independently of the surrounding, and is induced by the activation of caspases, specific endoproteases that destroy the essential structural components including the genetic material of the cell [9]. Caspase-3 (cas-3), due to its specificity and sensitivity, is a reliable marker of cells that pass the process of programmed cell death. Its activity cannot be detected before apoptosis. It is registered in early stages and detection grows with progression while is reduced only in the final phase of the apoptotic process [10].

Furthermore, angiogenesis is mediated by numerous angiogenetic growth factors; the most powerful among them is the vascular endothelial growth factor (VEGF). VEGF affects the degree of microvascular tumor density by stimulating the proliferation of endothelial cells. Variable concentrations of VEGF, depending on the phase of menstrual cycle, are detected in myometrium, stromal and epithelial endometrial elements [11].

During tumor angiogenesis, endothelial cells intensely express endoglin (CD105), while vascular endothelium, stromal and inflammatory cells barely or do not express at all CD105 [12].

Beside the fact that the prevalence of proliferation over apoptosis is a major condition for myoma growth, with this study we tried to indicate that similarities and differences in angiogenesis, proliferative, and apoptotic indexes in myomas and surrounding tissue, are primarily dependent on the expression of steroid hormone receptors. According to previous, the aim of this study was to determine the effects of the ER and PR status on angiogenesis, proliferation, and apoptosis of myoma cells in premenopausal (PreM) and postmenopausal (PostM) women.

METHODS

This was a cross section; clinical-experimental, retrospective, non-interventional study in the field of the study of fundamental pathogenesis mechanisms of disease using pathohistological materials from the existing archive.

The research included 76 patients diagnosed with uterine leiomyomas, operatively treated in the Clinic for Gynecology and Obstetrics, Clinical Centre Kragujevac, Kragujevac, Serbia, in a three-year-long period (2007–2010). According to the menstrual status, two experimental subgroups were formed. The first group was PreM women (n = 35; 46.2 ± 5.02 years old), and the second group was PostM women (n = 41; 60.25 ± 5.41 years old).

Clinical data were collected by insight into disease history and operational protocols of examined patients. We collected information related to gynecological status (menstrual cycle, menarche, menopause, number of deliveries, etc.) and data obtained by macroscopic analysis of the operative preparations (number, position and size of the myoma, changes in the ovaries, morphometric characteristics of the uterus).

The experimental part of the study was carried out on the operative tissue material obtained by hysterectomy.

The study was conducted at Department of Pathology, Clinical Centre of Kragujevac, Serbia. The study was done in accord with standards of the institutional Committee on Ethics of the Clinical Center of Kragujevac, Kragujevac, Serbia.

Hematoxylin-eosin staining

Tissue materials were fixed in formalin, embedded in paraffin, and 5-μm sections were stained with hematoxylin-eosin (H&E), and further examined by immunohistochemistry [13]. All pictures are taken in original resolution with ×200 magnification. A representative sample of the myoma without regressive changes is separated for immunohistochemical analysis.

Immunohistochemistry

Paraffin-embedded tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin using standard pathological protocols. Immunohistochemistry was performed on a single representative block from each case or two (when the surrounding myometrium is not visible along with the myoma on the first block). Antigenic retrieval was processed by submerging the sample in 10 mM citrate buffer (pH 6) or commercial buffer 10 mM EDTA Buffer for Heat-Induced Epitope Retrieval (pH 8), AP-9004-125 (Thermo Scientific, Waltham, MA, USA) and microwaving for 20 minutes at 96°C. Primary monoclonal antibodies were directed against ERα Ab11 (mouse: 1:500, MS-354-R7, Thermo Scientific), ERβ antibody (mouse/human: 1:200 dilution, MA1-23217, Thermo Scientific), VEGF (rabbit: 1:100 dilution, RB-9031-RQ, Thermo Scientific), Ki-67 (rabbit: 1:100 dilution, RB-9106-R7, Thermo Scientific), PRa Ab-8 (mouse: 1:25 dilution, MS-298-R7, Thermo Scientific), CD105 (rabbit: 1:25 dilution, RB-9291-R7, Thermo Scientific), and cas-3 Ab-3 (human: 1:100 dilution, MS-1123-R7, Thermo Scientific). Tissue sections were incubated with appropriate primary antibody and commercial biotinylated secondary anti-immunoglobulin, at room temperature, according to the manufacturer's instructions (UltraVision LP Large Volume Detection System: HRP Polymer (Ready-To-Use), TL-125-HL, Thermo Scientific). An evaluation of the immunohistochemical analysis was carried out by a semi-quantitative assessment of the expression of the examined markers, by scaling to the scales specific to each marker. All pictures are taken in original resolution with ×200 magnification.

Expression of estrogens, progesterone, vascular endothelial growth factor, and Ki-67

The expression of ER and PR will be quantified based on the Allred score, i.e. by adding parameters that indicate percentage representation (from 0 to 5) and intensity of cell

expression (from 1 to 3) [14]. The sum of these parameters will represent the values of the total score (from 0 to 8), where the values ≥ 3 was considered positive. The expression of VEGF, Ki-67 and cas-3 was determined based on the percentage of immunoreactive cells. Based on this expression, groups with low (0–15%), moderate (16–30%) and high proliferative or apoptotic index (31–100%) were formed.

Expression of endoglin

Immunohistochemical analysis of the expression of endoglin (CD105), an assessment of the degree of angiogenesis will be performed. The right index of intensity of angiogenesis is the density of intra and peritumoral microcirculation or microvessel density. The analysis will be carried out quantitatively by counting blood vessels in zones with their highest density (hot spot areas). We used the recommendations given by Weidner et al. [15] on the magnitude of the field of vision and the counting method. The focal points of the highest density of blood vessels were determined on a small microscopic magnification (×40). Determination of the focus of the largest microvascular density were performed by two researchers independently, with no clinical and pathohistological data available. After that, the counting of individual blood vessels was performed at a mean microscopic magnification (×200), which implies an area of 0.739 mm^2. The mean value of the results obtained by counting in three visible fields was the result. When counting blood vessels in each "hot zone," the expression of individual endothelial cells, and not just the lumen of a blood vessel with visible red blood cells, was calculated. After obtaining the data on the number of blood vessels for each case separately, the mean value of the three read fields were calculated. Then the median in relation to which all myomas were classified into two groups, those with low degree and those with a high degree of angiogenesis, accordingly whether the number of blood vessels is less than or equal to or greater than the value of the calculated median.

All immunohistochemical staining were carried out with quality control and specificity of colouring, using positive and negative controls according to the UK National Quality Assessment for Immunocytochemistry. Microscopic tumor analysis and evaluation of marker expression were performed on a microscope of the Carl Zeiss, Axioscop 40 type. Preparations with representative fields were painted using three microscopic enlargements (×40 and ×200) using a Canon PC 1089 camera (Canon Inc., Tokyo, Japan).

Sampling

Regarding the method of selecting a study sample from the entire population, all samples of the material archive will be potentially considered for inclusion. The criteria for the involvement of subjects in the study were a pathohistologically verified uterine leiomyomas disease and PreM or PostM status. Excluding criteria for selecting subjects were associated malignant diseases of the ovary and cervix, incomplete clinical data on menstrual status, use of oral contraceptives and other forms of hormonal therapy.

Statistical analysis

Statistical processing of results will be performed using a commercial software package SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). In the analysis of the obtained results, descriptive statistics was first used to describe the general characteristics of the sample: absolute numbers and proportions (frequencies, percentages), median and variability measures (standard deviation), maximum and minimum. The regularity of the distribution was evaluated by Kolmogorov–Smirnov test. For the comparison of the mean values of the two variable populations, the independent t-test, Kruskal–Wallis and Mann–Whitney tests were used, and for comparison of the mean variables of populations analysis of variance was used. The dependence of two descriptive variables were carried out using the χ^2 test and the Fisher test, the dependence of two numerical variables using Pearson's and Spearman's correlation coefficient, while the influence of more variables on the binary variable were investigated using a multivariate binary logistic regression.

RESULTS

Hematoxylin staining

The standard light microscopic analysis is defined histological type of myoma, the mitotic index expressed through the number of mitotic figures on 10 fields of great enlargement, the presence of regressive changes (necrosis, hyaline and myxomatous degeneration, foci of hemorrhage, etc.), the condition of the endometrium and surrounding myometrium (Figure 1A, B).

Expression of estrogen receptors alpha and beta in myoma and myometrium

Neither ERα nor ERβ showed statistically significant expression in myoma of PreM compared to PostM women (Figures 2A, B and Figures 2E, F). Similarly, those parameters were not different among examined groups in myometrium samples (Figures 4A, B and Figures 4E, F).

Expression of progesterone receptor in myoma and myometrium

PR was over expressed in myoma of PreM compared to myoma of PostM women (Figure 2C and 2G). Also, statistically significant increased values of PRa were detected in myometrium of PreM compared to PostM women (Figure 4C and 4G).

Expression of the vascular endothelial growth factor in myoma and myometrium

In myoma tissue, VEGF was not significantly different among groups (Figure 1D and 1H). Similarly, the same result was obtained in myometrium of PreM women compared to PostM women (Figure 4D and 4H).

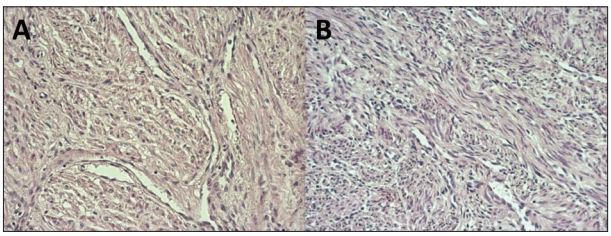

Figure1. A: representative image of a human myoma (H&E, magnification: ×200); B: representative image of a human myometrium (H&E, magnification: ×200)

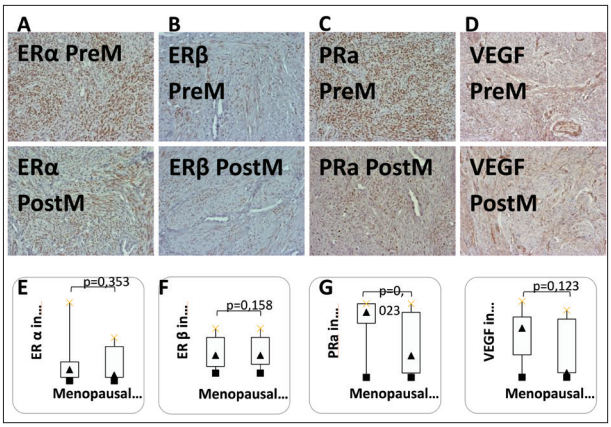

Figure 2. A–D: immunohistochemical expression of ERα, ERβ, PRa, and vascular endothelial growth factor (magnification: ×200) in myoma tissue of premenopausal and postmenopausal women, representative tissue sections; E–H: percentage of expression of ERα, ERβ, PRa, and vascular endothelial growth factor and statistical difference in percentage of myoma cells between premenopausal and postmenopausal women (▲ – median; ■ – minimum; × – maximum)

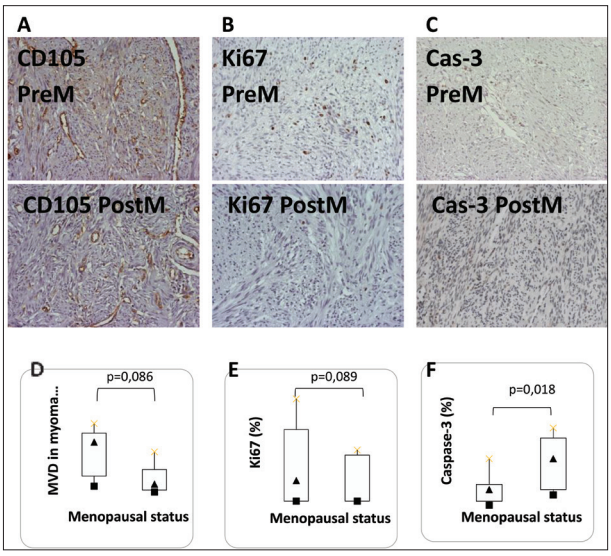

Figure 3. A–C: immunohistochemical expression of endoglin, Ki67, and Cas-3 (magnification: ×200) in myoma tissue of premenopausal and postmenopausal women, representative tissue sections; D–F: percentage of expression of endoglin, Ki67 and Cas-3, and statistical difference in percentage of myoma cells between premenopausal and postmenopausal women (▲ – median; ■ – minimum; × – maximum)

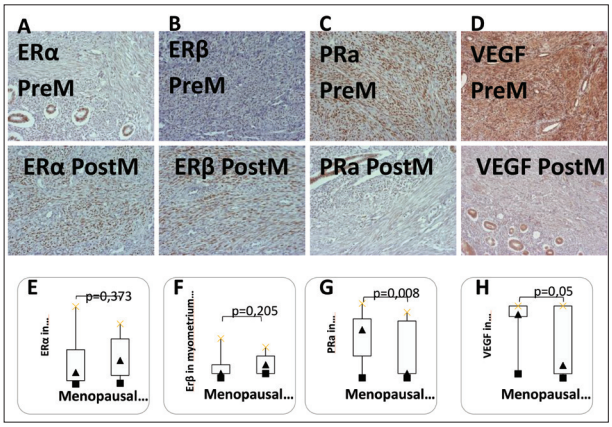

Figure 4. A–D: immunohistochemical expression of ERα, ERβ, PRa, and vascular endothelial growth factor (magnification: ×200) in myometrium tissue of premenopausal and postmenopausal women, representative tissue sections; E–H: percentage of expression of ERα, ERβ, PRa, and vascular endothelial growth factor and statistical difference in percentage of myometrium cells between premenopausal and postmenopausal women (▲ – median; ■ – minimum; × – maximum)

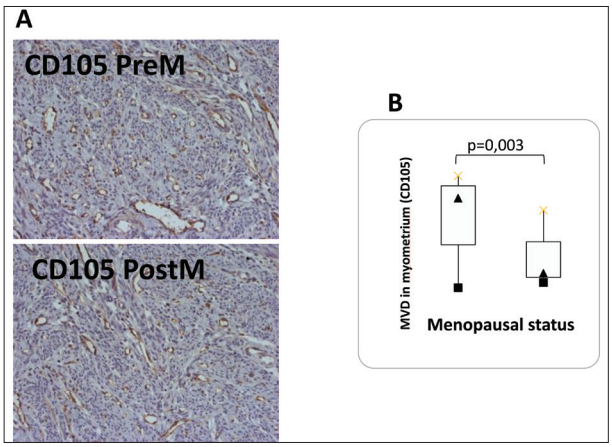

Figure 5. A: immunohistochemical expression of endoglin, (magnification: ×200) in myometrium tissue of premenopausal and postmenopausal women, representative tissue sections; B: percentage of expression of endoglin and statistical difference in percentage of myometrium cells between premenopausal and postmenopausal women (▲ – median; ■ – minimum; × – maximum)

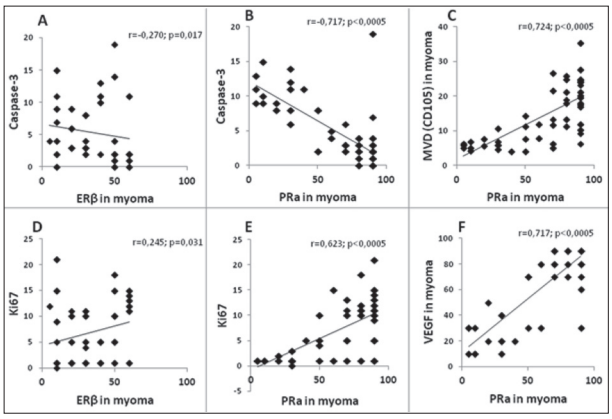

Figure 6. Correlation between all examined parameters in myoma tissue

Expression of endoglin in myoma and myometrium

In myoma of PreM and PostM women CD105 did not show any difference in its expression (Figure 3A and 3D). However, in myometrium CD105 was statistically significant increase in PreM women compared to PostM women (Figure 5A and 5B).

Expression of Ki67 in myoma

Ki67 was not significantly different among PreM and PostM women (Figure 3B and 3E).

Expression of caspase-3 in myoma

Expression of cas-3was statistically significant increase in PostM women compared to PreM group (Figure 3C and 3F).

Correlation between all examined parameters in myoma tissue

Expression of cas-3 in myoma tissue of all examined groups was weak, but statistically significant negative correlation with expression of ERβ in the same tissue (Figure 6A). Moreover, expression of cas-3 was in strong and significant negative correlation with PRa in myoma tissue of both PreM and PostM women (Figure 6B).

In strong correlation with expression of PRa was expression of CD105, VEGF and Ki67 as it is showed in Figures (Figures 6C, E, and F). Moreover, expression of Ki67 was in weak but statistically significant correlation with expression of ERβ (Figure 6D) while cas-3 was in negative correlation with ERβ (Figure6A). Correlations between other examined parameters in tissue of myoma did not show to be significant.

DISCUSSION

Uterine leiomyoma is the most common benign tumor; despite its frequent manifestation the etiology and pathophysiology of this abnormality remain unknown. Extensive knowledge has accumulated on the role of hormones in the growth of leiomyomas because the occurrence of uterine leiomyomas during the fertile period and the regression after menopause indicate that gonadal steroids are central for development of these tumors [7, 16]. In the last decade, special attention was given to the role of estrogens and progesterone in the pathophysiology of leiomyomas. Uterine leiomyomas have been considered estrogen-dependent tumors, and his role was supported by the finding that continuous gonadotropin-releasing hormone agonist treatment, significantly decreases ovarian estrogen production, is as well associated with reduction in tumor size [17]. In order to achieve their effects, estrogens act trough the activation of estrogen receptors (ERα and ERβ). Both of these receptors exhibit DNA- and ligand-binding domain sequence conservation and they are encoded by two distinct genes, they also have different transcriptional activation domains, as well as different tissue distribution [17]. In our study, we showed that expressions of those receptors were not different in myoma of PreM compared to PostM women (Figure 1E, F). Nevertheless, expression of ERα and ERβ was not significantly different neither in myometrium of those women (Figure 3E, F). Similarly, Sakaguchi et al. [18] showed that coordinated expression of ERα and ERβ might be necessary for normal estrogen action in myometrium [19]. In addition, it has been shown ERα is phosphorylated at a higher rate on serine in leiomyoma compared with surrounding myometrium, for that reason it is possible that phosphorylated ERα regulated by p44/42 MAPK, will have a role in development of uterine leiomyoma [20, 21].

In recent years, the role of progesterone in uterine leiomyoma pathophysiology has become more established. As in the case of ERs, nuclear PR work as ligand-activated transcription factors and there is two predominant isoforms of PR in humans: PRa and PRb [17]. In our study, PRa showed to be significantly unregulated in myoma of PreM compared to myoma of PostM women, which showed to be same in myometrium tissue (Figure 1G, 3G). Those finding correlate with the fact that progesterone is cyclically elevated during the reproductive years, are significantly elevated during pregnancy, and are suppressed after menopause, however it is still very difficult to distinguishing the relative importance of estrogen versus progesterone [22, 23].

Since it is already a historical fact that leiomyomas are dependent on angiogenesis for their growth and survival, to date it is also found that estrogens and progestins regulate the expression of several potent angiogenic factors, including VEGF and fibroblast growth factor (FGF) [24]. We found that in myoma VEGF was not significantly changed regardless of the menopausal status (Figure 2H); in contrast, VEGF was changed between groups in myometrium (Figure 4H). We found that VEGF is significantly increased in PreM compared to the PostM women. Similarly, Hague et al. [25], according to menopausal status found that VEGF was significantly increased in PreM compared with PostM endometrium. With the PreM tissue, exhibiting a significantly higher level of expression was found in the epithelium but not in the stroma or the blood vessels [26].

Furthermore, one of the most commonly assessed angiogenesis markers is microvessel density, which is determined on the bases of specific endothelial antigen expression (CD34, CD105) [27]. In the tumor we did not notice any change between groups in expression of CD105 regarding menopausal status (Figure 3D), however CD105 was statistically significantly decreased in myometrium of PostM compared to PreM women (Figure 5B).

Moreover, regarding cell death, we evaluated two proteins known as markers involved in growth control of leiomyoma. There was no difference between two examined groups in expression of Ki67 (Figure 3E); however, cas-3 was significantly increased in PostM compared to PreM women (Figure 3F). We noticed that expression of Ki67 did not follow the trend of cas-3 expression, regarding that Plewka et al. [7] showed that the apoptosis was not accompanied by proliferation. There were no immunolocalization of Ki-67 detected in leiomyomas manifesting apoptosis [28].

Additionally, as we can see from Figure 6, PRa is in positive correlation with VEGF, CD105 and Ki67 (Figure 6C, E and F) in myoma cells, and in negative correlation with cas-3 (Figure 6B). On the other hand, ERβ was in negative correlation with cas-3 and in positive correlation with Ki67 (Figure 6A, D).

CONCLUSION

Although ERβ have effect on cell proliferation and apoptosis, according to all the data, PRa seems to be more relevant. According to our data, PRa had higher influence on apoptosis and cell growth then ER. Since PRa was increased in PreM in both myoma and myometrium, this expression probably led further to lower expression of the apoptotic marker, increased cell proliferation, and angiogenesis in PreM women.

Further studies need to be conducted in order to better understand the mechanisms associated with progesterone-driven growth, according to our data, in order to develop therapies that are more efficient.

Conflict of interest: None declared.

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Утицај експресије стероидних рецептора на ангиогенезу, пролиферацију и апоптозу у миомима пременопаузних и постменопаузних жена

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САЖЕТАК

Увод/Циљ Циљ ове студије је био да се утврди утицај експресије стероидних рецептора на маркере ангиогенезе, пролиферације и апоптозе ћелија миома код жена у пременопаузи и постменопаузи.

Методе Ово је била студија пресека, клиничко-експериментална, ретроспективна, неинтервенцијска студија у пољу истраживања фундаменталних механизама патогенезе болести, коришћењем патохистолошких материјала из постојеће архиве. Истраживањем је обухваћено 76 болесника са дијагностикованим лејомиоима утеруса, оперативно лечених на Клиници за гинекологију и акушерство Клиничког центра Крагујевац, Србија. Према менструалном статусу, формиране су две експерименталне подгрупе. Прва група биле су жене у пременопаузи (n = 35; 46,2 ± 5,02 година), а друга група биле су жене у постменопаузи (n = 41; 60,25 ± 5,41 година). Коришћено је бојење *H&E* за миом и миометријум, као и имунохистохемија за *ERα*, *ERβ*, *PR*a, васкуларни ендотелни фактор раста, ендоглин, *Ki*67 и *caspase* 3.

Резултати Прогестеронски рецептор је био више изражен у миому и миометријуму у пременопаузи у поређењу са миомом и миометријумом жена у постменопаузи. Експресија *caspase-3* је статистички значајно повећана у групи жена које су у постменопаузи у поређењу са групом жена које су у пременопаузи. *ER*α и *ER*β нису били различити имеђу група ни у узорцима миома ни миометријума.

Закључак Према нашим подацима, *PR*a је имао већи утицај на апоптозу и раст ћелија него рецептори за естрогене. Пошто је *PR*a повећан код жена у пременопаузи у миому и миометријуму, вероватно је ова експресија довела до смањења експресије апоптотског маркера код жена које су у пременопаузи.

Кључне речи: рецептори за стероиде; апоптоза; ангиогенеза; пременопауза; постменопауза

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Dependence of the allergic status markers on the level of vitamin D in the serum

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SUMMARY

Introduction/Objective Recent researches show a link between low vitamin D serum levels and increased prevalence of allergic disease.

The objective of this study was to show whether there is any dependence of the allergic status markers: skin prick test (SPT), total immunoglobulin E IgE (tIgE), and allergen-specific IgE (sIgE ≥ 3 class) in serum from the serum 25(OH)D (vitDs) level in children with allergic disease/s.

Methods A total of 150 children with allergic disease/s were enrolled into this study. The vitDs, tIgE, SPT, and sIgE ≥ 3 class for aeroallergens and common food allergens were simultaneously assessed.

Results We found a negative correlation between vitDs level and age groups and a statistically significant positive correlation between vitDs level and tIgE, sIgE ≥ 3 class for hen's egg yolk and hen's egg white. A statistically significant positive correlation was determined between vitDs level and SPT on *Dermatophagoides pteronyssinus*, and a negative correlation between tIgE and SPT on *Dermatophagoides pteronyssinus*, as well as between vitDs level and sIgE ≥ 3 class for *Cladosporium* and *Alternaria* molds. We confirmed the dependence of nettle rash and comorbidity asthma from the vitamin D insufficiency and vitamin D deficiency. We did not find any dependence of serum tIgE on vitDs level for the sample.

Conclusion In order to get an adequate insight into the allergic status in children, we must take into account the pleotropic effects of vitamin D, according to which we suggest that, in the future, vitDs level should be determined synchronously with known markers of allergic status.

Keywords: immunoglobulin E; vitamin D; child; allergen

INTRODUCTION

An allergy is a disorder caused by an abnormal reaction to a harmless substance called an allergen. An allergy may manifest itself as a food allergy, atopic dermatitis, allergic asthma, allergic rhinitis, allergic conjunctivitis, and urticaria. The prevalence of allergic disease has increased considerably during the last decades. About 30% of the population in Europe is "attacked by allergies;" the situation with children is alarming – every third child suffers from at least one allergic disease.

Considering the pleiotropic effects of vitamin D (especially on the development of immune system tolerance and of the integrity of the epithelial barrier), recent studies have hypothesized a correlation between vitamin D and the rising incidence of allergic disease.

Markers of allergic status – skin prick test, total and specific immunoglobulin E

Allergy skin prick test (SPT) is the gold standard for confirmation of immunoglobulin E (IgE)-mediated allergic diseases. SPT is well reproducible, easy to perform, reliable, very safe, and more sensitive than allergen-specific IgE (sIgE) [1]. SPT imperfections are many: it is difficult to compare results from different countries because they use different extracts, training of staff and parents is required, it takes a long time to perform, and in some countries SPT is considered less safe than sIgE for certain allergens. Serum sIgE emerges as an alternative test in the field of allergy diagnosis. In some countries, for reasons of conformity, it is resorted to an estimate of the atopic state in young children solely by measuring the level of sIgE (circulating IgE) for certain allergens in the serum [2].

A link between vitamin D serum levels and an increased prevalence of allergic diseases has been proposed. Results of the National Health and Nutrition Examination Survey for 2005–2006 determined a consistent association between 25(OH)D deficiency and higher levels of IgE sensitization in children and adolescents [3].

However, there are not many studies that evaluate the correlation between serum 25(OH)D level (vitDs) and the markers of allergic status (SPT, tIgE, sIgE) in children with allergic disease/s. Since tIgE is considered a good predictor of allergy in children, and SPT and allergen sIgE are the most widely used diagnostic tests in allergy, we observe the association, correlation, and dependence between them (SPT, tIgE, sIgE) and vitDs level.

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Vitamin D and immunomodulation related to allergy

The potential role of vitamin D on the immune system is described after the discovery of VDR on macrophages, dendritic cells, activated B and T lymphocytes, as well as the ability of these cells to express 1-α-hydroxylase [4]. Up-regulation of 1-α- hydroxylase in DC is associated with the maturation process of these cells, suggesting that local production of 1,25(OH)D might serve as negative feedback to prevent inflammation. Vitamin D inhibits the expression of inflammatory cytokines and interferons in monocytes (IL-1, IL-6, IL-8, IL-12, TNF-α). Also, vitamin D affects the cells of the humoral immune response; it inhibits the proliferation and differentiation of B cells, thereby indirectly affecting the synthesis of immunoglobulins [5, 6, 7].

The objective of this study was to show whether there is any dependence of the allergic status markers (SPT, tIgE, sIgE) on serum vitDs level in children with an allergic disease.

METHODS

A total of 150 children with allergic disease were included in the study to investigate the association and dependence between the vitDs level and the markers of allergic status (SPT, tIgE, sIgE). The study was conducted with permission of the institutional ethics committee (01-6917/23.05.2016), at the Clinic of Pediatrics (PC), Kragujevac Clinical Center, Serbia, from January 2014 to June 2016.

The main criteria for patients included in the study were as follows: 1) age: 0–18 years; 2) suffering from at least one of the following diseases: asthma, allergic rhinitis, atopic dermatitis, urticaria, food allergies; the diagnosis was made according to the criteria defined by the protocols of Global Initiative for Asthma and Allergic Rhinitis and its Impact on Asthma [9], and of the World Allergy Organization [8, 9, 10]; for the classification of children, we used the diagnosis with which the children were discharged; 3) tIgE; 4) vitDs; 5) SPT; 6) sIgE with cut-off class three (sIgE ≥ 3 class).

Allergy skin prick test

We used allergen extract solutions manufactured by Torlak (Belgrade, Serbia) for seven aeroallergens (animal hair – cat's and dog's hair, molds, mix of tree pollens, mix of ragweed pollens, house dust mites, cockroach) and six food allergens (hen's egg yolk, hen's egg white, cow's milk, wheat flour, soybean, peanut). The test was performed according to the European standard for SPT to inhaled and nutritive allergens and positive/negative control [histamine dihydrochloride (10 mg/ml) / physiological sodium chloride (9 mg/ml)] [11]. Positive SPT was defined as a wheal diameter ≥ 2 mm above the negative control for children aged 0–3 years, and wheal diameter ≥ 3 mm for the children aged four years or older.

Specific immunoglobulin E in serum

The sIgE level was determined by using the AlleisaScreen (Mediwiss Analytic GmbH, Moers, Germany) screening method that is an immunoblot quantitative assessment of circulating allergen-specific IgE in serum. Tests were performed for matched panel of 17 aeroallergens (cat and dog hair E1–E5, *Cladosporium* and *Alternaria* M2–M6, *Penicillium–Aspergillus* M1–M3, maple pollen T1–T11, poplar T14, alder T2, birch T3, beech T5, ash T15, ragweed pollen W1–W2, *Dermat. pteronyssinus* D1, and cockroach I6) and eight food allergens (hen's egg yolk F75, hen's egg white F1, wheat flour F4, soybean F14, peanuts F13, lactalbumin alpha F76, lactalbumin beta F77, casein F78). The sIgE level ≥ 3.5 IU/ml or ≥ 3 class for certain allergens was adopted as an indicator of convincing allergic sensitization.

Serum measurements of total IgE and vitamin D

Total serum IgE was determined by using the electrochemiluminescence immunoassay (Cobas E 411) and was constituted in IU/ml. Measurements of vitamin D level was performed using electro-chemiluminescence binding assay (ECLIA) for the in-vitro determination of total 25(OH)D on Cobas® e 601 analyzer (Roche Diagnostics, Mannheim, Germany). VitDs were categorized into three vitamin D statuses: sufficient (≥ 30 ng/ml), insufficient (20–30 ng/ml), and deficient (< 20 ng/ml) [12].

Statistical analysis

Statistical data processing was performed using IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA). We used descriptive statistical methods for continuous variables: mean, standard deviation. The correlations were assessed by Spearman's rank correlation test. In order to test the hypothesis of the mean values, we used nonparametric tests, Mann–Whitney U test for comparisons between two groups, and Kruskal–Wallis test for comparing three or more groups, followed by Bonferroni post-hoc test for multiple comparisons between subgroups. P-values < 0.05 were considered statistically significant.

RESULTS

Out of 150 patients enrolled in this study, 86 were male (56%) and 66 (44%) were female. The age groups ranged from one month to 17 years, with the mean age being 7.11 ± 3.8 years. Forty-seven (31.7%) patients had medical history positive for allergic disease in the mother and 33 (22%) in the father; 122 patients (81.3%) had positive SPT; 86 (57.3%) patients had increased serum sIgE ≥3 class for at least one of the tested allergens. The mean value of total serum IgE was 327.42 ± 533.56 IU/ml. The mean level of vitDs was 20.44 ± 8.26ng/ml. Established vitamin D statuses were deficiency (< 20 ng/ml) in 56% of the patients with the mean value of 14.46 ± 3.5, followed by insufficiency (20–30 ng/ml) in 31.3% of the patients with the mean value

being 24.88 ± 2.9, and sufficiency (> 30 ng/ml) in 12.7% of the patients, with the mean value being 35.87 ± 4.

Table 1 represents the mean value of tIgE and vitDs according to the age groups. We found a statistically significant difference in vitDs level between the age groups (p = 0.009). Also, there was a statistically significant difference in tIgE between the age groups (p = 0.004).

Table 1. Mean value of total immunoglobulin E (IgE) and vitamin D according to age groups

Age group (years)	Total IgE (IU/ml)	Vitamin D (ng/ml)
0–12 months (n = 7; 4.7%)	71.09 ± 96.8	35.38 ± 10.3
13–24 months (n = 18; 12%)	131.89 ± 314.6	23.24 ± 10.5
3–5 years (n = 26; 17.3%)	199.64 ± 263.2	20.10 ± 8.1
6–11 years (n = 79; 52.7%)	399.64 ± 64	18.94 ± 6.7
12–18 years (n = 20; 13.3%)	473.97 ± 520.2	19.02 ± 5.6

Table 2 shows the correlation between the age groups, tIgE, and vitDs level. We found significant positive correlation between the age groups of the participants and tIgE (p = 0.000). Also, we found a negative correlation between the vitDs level and age groups of the participants (p = 0.030).

Table 3 represents the correlation between vitamin D status in patients with allergic disease and one of the markers of allergic status. There we can see that there is a significant positive correlation between vitamin D status and SPT to aeroallergens (p = 0.016). Also, we found significant positive correlation between vitamin D status and sIgE ≥ 3 class to aeroallergens (p = 0.004).

In consideration of immunomodulatory effects of vitamin D in allergic disease, we observed the correlation between tIgE and vitDs level in patients with allergic disease. In our study we found a significant negative correlation between tIgE and vitDs level in patients with nettle rash (p = 0.000) and in patients with comorbidity asthma with atopic dermatitis (p = 0.000). Results of correlation between vitDs level and tIgE level in allergic diseases are shown in Table 4.

Table 2. Correlation between age groups (years), total immunoglobulin E (IgE) and serum 25(OH)D level

Parameter	total IgE (IU/ml)	Correlation between age in years and total IgE		Vitamin D (ng/ml)	Correlation between age in years and vitamin D	
		*rho	p		*rho	p
Age, years	327.42 ± 533.56	0.314**	0.000	20.44 ± 8.2	-0.178*	0.030

*p < 0.05;
**p < 0.001

Table 3. Correlation between vitamin D status and one of allergy screening tests

Allergy screening tests	Vitamin D status			Statistical correlation	
	Deficiency (n = 84)	Insufficiency (n = 47)	Sufficiency (n = 19)	*rho	p
Positive skin test to food allergens (n = 71)	45 (53.6%)	18 (38.3%)	8 (42.15%)	0.130	0.112
Negative skin test to food allergens (n = 79)	39 (46.4%)	29 (61.7%)	11 (57.9%)		
Positive skin test to aeroallergens (n = 106)	65 (77.4%)	32 (68.1%)	9 (47.4%)	0.196*	0.016
Negative skin test to aeroallergens (n = 44)	19 (22.6%)	15 (31.9%)	10 (52.6%)		
Positive sIgE to food allergens (n = 30)	17 (20.2%)	11 (23.4%)	2 (10.5%)	0.031	0.703
Negative sIgE to food allergens (n = 120)	67 (79.8%)	36 (76.6%)	17 (89.5%)		
Positive sIgE to aeroallergens (n = 79)	50 (59.5%)	28 (59.6%)	1 (5.3%)	0.234**	0.004
Negative sIgE to aeroallergens (n = 71)	34 (40.5%)	19 (40.4%)	18 (94.7%)		

Vitamin D statuses: sufficient (≥ 30 ng/ml), insufficient (20–30 ng/m), and deficient (< 20 ng/ml);
sIgE – allergen-specific IgE;
*p < 0.05;
**p < 0.001

Table 4. Correlation between serum 25(OH)D level and total immunoglobulin E (IgE) in allergic disease

Allergic disease	Parameters			
	Total IgE (IU/ml)	Vitamin D (ng/ml)	Spearman's correlation	p
Asthma	27.95 ± 14.2	26.17 ± 10.7	0.700	0.188
Allergic rhinitis	127.99 ± 305.2	22.16 ± 8.6	-0.277	0.251
Atopic dermatitis	45.53 ± 75.1	31.16 ± 11.6	0.607	0.148
Nettle-rash	181.37 ± 279.7	20.0 ± 3.6	-1.000**	0.000
Food allergy	55.85 ± 78.2	22.06 ± 6.7	0.100	0.873
Asthma + comorbidities				
Allergic rhinitis	253.99 ± 461.4	18.70 ± 6.7	-0.009	0.951
Atopic dermatitis	267.76 ± 333.4	21.55 ± 14.1	-1.000**	0.000
Allergic rhinitis + atopic dermatitis	466.16 ± 560.9	21.10 ± 10.3	0.273	0.446
Rhinitis allergic + food allergy	538.0 ± 665.1	19.05 ± 7.3	-0.067	0.643

**p < 0.001

Table 5. Features and findings in children suffering from allergic disease/s

Allergic disease/s	Sex M/F	Total IgE (IU/ml)	Vitamin D (ng/ml)	Specific IgE (IU/ml)													
				Aeroallergens					Food allergens							Skin Prick Test	
				D1	E1–E5	M2–M6, M1–M3	TP	I6	F2	F75	F1	F4	F14	F13	Aeroallergens P/N	Food allergens P/N
Asthma	4/1	27.95 ± 14.2	26.17 ± 10.7	0.03 ± 0.06	0.00	0.00	0.00	0.00	0.01 ± 0.0	0.00	0.00	0.00	0.00	0.00	3/2	3/2
Allergic rhinitis	11/8	127.99 ± 305.2	22.16 ± 8.6	1.74 ± 5.9	0.43 ± 1.5	0.87 ± 3.2	2.49 ± 10.4	0.52 ± 2.0	0.15 ± 0.2	0.02 ± 0.0	0.05 ± 0.1	0.79 ± 3.2	0.27 ± 1.0	0.62 ± 2.2	10/9	3/16
Atopic dermatitis	3/4	45.53 ± 75.1	31.16 ± 11.6	0.00	0.03 ± 0.0	0.05 ± 0.1	0.02 ± 0.06	0.00	0.10 ± 0.1	0.00	0.16 ± 0.4	0.42 ± 0.9	0.00	0.09 ± 0.2	4/3	4/3
Nettle rash	2/1	181.37 ± 279.7	20.0 ± 3.6	0.05 ± 0.0	0.09 ± 0.1	0.15 ± 0.2	0.06 ± 0.1	0.00	0.37 ± 0.6	0.01 ± 0.0	0.95 ± 1.5	0.03 ± 0.0	4.5 ± 7.9	0.62 ± 1.0	0/3	0/3
Food allergy	1/4	55.85 ± 78.2	22.06 ± 6.7	0.43 ± 0.6	0.11 ± 0.2	0.23 ± 0.2	0.04 ± 0.0	0.00	0.11 ± 0.2	0.02 ± 0.0	0.28 ± 0.6	0.58 ± 1.3	1.97 ± 4.3	0.38 ± 0.5	4/1	3/2
Asthma + comorbidities																
Allergic rhinitis	25/21	253.99 ± 461.4	18.70 ± 6.7	6.39 ± 14.0	0.15 ± 0.7	0.99 ± 4.3	1.04 ± 4.4	0.26 ± 1.0	0.26 ± 0.4	0.41 ± 2.6	0.17 ± 0.4	0.49 ± 1.9	0.39 ± 1.9	4.43 ± 20.6	33/13	21/25
Atopic dermatitis	1/1	267.76 ± 333.4	21.55 ± 14.1	7.85 ± 11.1	0.00	0.00	0.00	0.00	0.10 ± 0.1	0.00	0.50 ± 0.7	0.00	0.07 ± 0.0	0.70 ± 0.0	2/0	1/1
Allergic rhinitis + atopic dermatitis	6/4	466.16 ± 560.9	21.10 ± 10.3	5.90 ± 7.4	2.12 ± 6.0	1.34 ± 3.3	0.08 ± 0.2	0.00	0.27 ± 0.4	0.01 ± 0.0	10.02 ± 31.6	0.02 ± 0.0	0.00	0.74 ± 2.3	6/4	5/5
Allergic rhinitis + food allergy	31/22	538.0 ± 665.1	19.05 ± 7.3	11.65 ± 20.9	0.48 ± 1.9	1.19 ± 3.9	1.05 ± 2.9	0.16 ± 0.4	0.31 ± 1.0	0.44 ± 2.5	0.63 ± 2.7	0.42 ± 1.2	0.14 ± 0.3	2.43 ± 8.4	44/9	31/22

Aeroallergens: *Derm. pteronyssinus* – D1, animal hair (dog, cat) – E1–E5, mold (*Cladosporium–Alternaria* – M2–M6, *Penicillium–Aspergillus* – M1–M3), tree pollen – TP; insect (cockroach) – I6; food allergens: milk – F2 (F76, F77, F78), hen's egg yolk – F75, hen's egg white – F1, wheat flour – F4, soybean – F14, peanuts – F13;
IgE – immunoglobulin E

Table 5 represents the frequency of different allergic diseases in our patients with vitDs level, tIgE level, sIgE ≥ 3 class on different allergens and SPT on different allergens. Also, we did not find significant difference in the incidence of some allergic disease between boys and girls (p = 0.953). There is significant difference of tIgE level in different allergic diseases (p = 0.000). When it comes to aeroallergens, we observed that most of the patients were highly sensitive (sIgE ≥ 3 class) to dust mite, mold, and tree pollens. The analysis of the children's sensitivity to mold from the air (*Cladosporium–Alternaria* – M2–M6, *Penicillinum–Aspergilus* – M1–M3) is shown as the common result.

As regards food allergens, most of the patients were highly sensitive to soybean and peanuts. Also, we can consider an increased expression of sIgE in patients with allergic rhinitis (alone) as well as in patients with comorbidity asthma with allergic rhinitis, followed by comorbidity asthma with allergic rhinitis and atopic dermatitis, and comorbidity asthma with allergic rhinitis and food allergy. Also, we observed that the patients who had asthma with the associated allergic disease manifested highly sensitivity confirmed by SPT. There was no significant difference in vitDs level in different allergic diseases (p = 0.149). At the same time, there was a significant difference in vitDs level among children who had only one allergic disease and those with asthma comorbidity (p = 0.005). The mean serum 25(OH)D level in children who had only one allergic disease was 24.15 ± 9.3 ng/ml. Contrary to this, children with asthma comorbidity (with one or more allergic diseases) had lower mean serum 25(OH)D level of 19.13 ± 7.4 ng/ml.

Table 6 provides the correlation analysis between serum tIgE(IU/ml) and sIgE ≥ 3 class on certain aeroallergens and between sIgE ≥ 3 class on certain aeroallergens and vitDs level. We found a significant positive correlation between serum tIgE(IU/ml) and sIgE ≥ 3 class on the following allergens: *Derm. pteronyssinus* (p = 0.004), mold *Penicillinum–Aspergilus* (p = 0.001), tree pollens – ash tree (p = 0.001), and cockroach (p = 0.041), as between serum sIgE ≥ 3 class on animal hair (cat and dog) and vitDs level (p = 0.008). The negative correlation determined between vitDs level and serum sIgE ≥ 3 class on *Cladosporium–Alternaria* mold (p = 0.001).

Table 7 represents the correlation analysis between serum tIgE and sIgE ≥ 3 class on food allergen as between serum sIgE ≥ 3 class on food allergen and vitDs level. Here, we found the statistical positive correlation between serum tIgE and sIgE ≥ 3 class on certain food allergen for hypersensitivity on: alfa-lactoglobulin (p = 0.007), hen's egg yolk (p = 0.000), hen's egg white (0.048). Likewise, we found a significant positive correlation between vitDs level and serum sIgE ≥ 3 class on hen's egg yolk (p = 0.000) and hen's egg white (0.050).

We found a significant negative correlation between serum tIgE and SPT on *Derm. pteronyssinus* (p = 0.000) and tree pollen (p = 0.001), as shown in Table 8. We did not find significant correlations between serum tIgE and SPT on food allergens. We presented these results in Table 9. By analysis, we found positive correlation and

Table 6. Correlation between total and allergen-specific immunoglobulin E (IgE) > 3 class on certain aeroallergens, as well as sIgE > 3 class on certain aeroallergens and serum 25(OH)D level

Aeroallergens	sIgE (IU/ml) > 3 class	Serum total IgE (IU/ml) level	Correlation total IgE *vs.* sIgE		Vitamin D (ng/ml)	Correlation vitamin D *vs.* sIgE > 3 class	
			*rho	p		*rho	p-value
Dermatophagoides pteronyssinus (n = 38)	24.91 ± 20.8	674.40 ± 765.48	0.813**	0.004	18.33 ± 6.8	-0.104	0.527
Animal (n = 6)	9.03 ± 5.7	948.45 ± 631.62	0.353	0.493	16.57 ± 5.1	0.884**	0.008
Cladosporium–Alternaria (n = 14)	16.01 ± 15.5	618.31 ± 690.7	-0.103	0.725	18.39 ± 8.8	-0.699**	0.001
Penicillinum–Aspergilus (n = 2)	8.65 ± 6.3	581.07 ± 807.6	1.000**	0.001	24.85 ± 3.8	-1.000	/
Tree pollen							
Maple (n = 8)	22.23 ± 27	629.48 ± 470.6	-0.253	0.545	19.39 ± 6.5	0.157	0.711
Poplar (n = 3)	8.23 ± 5.7	529.09 ± 514.5	1.000	/	16.89 ± 4.9	0.500	0.667
Alder (n = 9)	14.82 ± 11	644.54 ± 463.16	-0.193	0.618	21.23 ± 8.2	0.059	0.881
Birch (n = 11)	11.46 ± 7.1	571.90 ± 445.9	0.78	0.821	19.52 ± 8.3	0.196	0.563
Hazel bush (n = 12)	7.20 ± 10	760.52 ± 487.6	-0.310	0.327	21.41 ± 10	0.014	0.965
Beech (n = 11)	25.79 ± 28	608.14 ± 434.2	0.132	0.689	17.25 ± 6.6	0.562	0.072
Mix of ragweed (n = 7)	32.41 ± 23.4	681.98 ± 538	0.429	0.337	19.40 ± 6.3	-0.714	0.071
Ash tree (n = 2)	5.95 ± 2.7	601.41 ± 705.8	1.000**	0.001	19.57 ± 2.5	-1.000	
Cockroach (n = 3)	6.53 ± 2.2	529.09 ± 514.58	0.727*	0.041	16.89 ± 4.9	0.500	0.667

sIgE – allergen-specific IgE;
*p < 0.05;
**p < 0.001

Table 7. Correlation between total total and allergen-specific immunoglobulin E (IgE) > 3 class on certain food allergen, allergen-specific immunoglobulin > 3 class on certain food allergen and serum 25(OH)D level

Food allergens	sIgE (IU/ml) > 3 class	Serum total IgE (IU/ml) level	Correlation total IgE *vs.* sIgE		Vitamin D (ng/ml)	Correlation vitamin D *vs.* sIgE > 3 class	
			*rho	p		*rho	p
Milk (n = 0)	/	/	/	/	/	/	/
Alfa-lactoglobulin (n = 6)	5.35 ± 5.6	275.87 ± 343.6	0.993**	0.007	18.76 ± 5.6	0.029	0.957
Beta-lactoglobulin (n = 0)	/	/	/	/	/	/	/
Casein (n = 2)	10.12 ± 9	579.41 ± 809.9	/	/	18.50 ± 5	/	/
Hen's egg yolk (n = 2)	18.20 ± 0.5	579.41 ± 809.9	1.000**	0.000	15.50 ± 5	1.000**	0.000
Hen's egg white (n = 4)	32.50 ± 45.4	869.55 ± 552	0.949*	0.048	24.94 ± 10.7	0.949*	0.050
Wheat flour (n = 5)	9.28 ± 3.3	552.83 ± 365.31	0.053	0.933	20.50 ± 6	-0.684	0.203
Soybean (n = 4)	10.33 ± 4.1	344.91 ± 512.8	-0.800	0.200	20.24 ± 3	0.400	0.600
Peanuts (n = 9)	36.07 ± 39.3	923.99 ± 896.9	-0.33	0.932	20.91 ± 8.4	-0.435	0.242

sIgE – allergen-specific IgE;
*p < 0.05
**p < 0.001

dependence of only SPT to *Derm. pteronyssinus* from vitDs level (p = 0.050) and we did not find the correlation and dependence of other SPT to aero- and food allergens from vitDs level.

DISCUSSION

An increasing incidence of allergic disease during the past 30 years sets the need to seek laboratory parameters that are useful in diagnosing allergic disease. Park et al. [13] in their study showed that the serum total IgE level is a good predictor of allergy in children. Several papers indicated a problem of discrepancy between the results obtained with an SPT and allergen sIgE. Schoos et al. [14] determined poor or moderate degree of agreement between the results obtained from SPT and sIgE for a certain allergen, which shows that this ratio deteriorates with age of the child. According to these Norwegian authors, it is necessary to use complementary SPT and allergen sIgE, but not interchangeably, especially in young children (0–2 years) [14]. Regarding the role of vitamin D in the regulation of the immune system, vitamin D status can be one of the effective factors in the reactivity of a certain allergen. Studies conducted by Kolokotroni et al. indicated that serum level of vitamin D is positively associated with tIgE level and sIgE on *Dermatophagoides farinae* in Cyprus children [15]. In our study, we found a negative correlation between vitDs level and tIgE and sIgE ≥ 3 class to aeroallergens, as well as between vitDs level and sIgE ≥ 3 class on mold *Cladosporium–Alternaria*, and, finally, a statistically significant positive correlation between vitDs level and sIgE ≥ 3 class to animal hair (cat and dog), which we consider to be interdependence.

Likewise, related to food allergens, we found a statistically significant positive correlation between vitDs level and tIgE, and sIgE ≥ 3 class on hen's egg yolk and hen's egg white (p = 0.006), which we consider to be interdependence.

Table 8. Correlation between skin prick test (SPT) on aeroallergen and total immunoglobulin E (IgE), as well as that of SPT on aeroallergens and serum 25(OH)D level

SPT for aeroallergens	Total IgE *vs*. SPT		Vitamin D *vs*. prick test	
	*rho	p	*rho	p
Derm. pteronyssinus (n = 58)	-0.359**	0.000	0.157*	0.050
Mold (n = 19)	-0.059	0.477	-0.071	0.386
Animal (n = 9)	0.065	0.433	-0.091	0.270
Tree pollen (n = 46)	-0.273**	0.001	-0.015	0.852
Cockroach (n = 8)	0.082	0.317	0.095	0.246

*p < 0.05;
**p < 0.001

Table 9. Correlation between skin prick test (SPT) on food allergen and total IgE, as well as that of SPT on food allergens and 25(OH)D level

SPT for food allergens	Total IgE vs. SPT		Vitamin D vs. SPT	
	*rho	p	*rho	p
Milk (n = 12)	0.122	0.137	0.091	0.271
Hen's egg yolk (n = 12)	-0.083	0.313	-0.094	0.253
Hen's egg white (n = 14)	-0.065	0.432	0.001	0.995
Wheat flour (n = 11)	0.062	0.453	0.097	0.237
Soybean (n = 4)	0.072	0.383	-0.079	0.335
Peanuts (n = 19)	-0.013	0.877	0.026	0.751

*p < 0.05
**p < 0.001

Several studies investigated the relationship between vitamin D deficiency and allergic diseases and concluded that low level of vitamin D is associated with increased incidence of allergies and asthma [16, 17, 18]. Poole et al. [19] in their study conducted on infants showed that vitamin D insufficiency is associated with an increased risk of a challenge-proven peanut/egg allergy. Quirk et al. [20] suggest that vitamin D deficiency increases the risk of sensitization to food allergens, particularly to milk and wheat. In our study, we found a statistically significant correlation between nettle rash and comorbidity asthma (with one or more allergic diseases) and lower mean vitDs level (p = 0.005), which we consider to be interdependence.

We found a statistically significant difference in serum vitD level according to SPT in children with allergic disease (p = 0.050). The mean value of 25(OH)D level in children with positive SPT was 19.77 ± 7.91 ng/ml in serum. The children with negative SPT had a mean value of 23.34 ± 9.2 ng/ml in serum 25(OH)D. From this result, we can remark that the high frequency of positive SPT (81.3%) in children with allergic disease means high frequency of vitamin D insufficiency and vitamin D deficiency, which leads us to conclude that there is a dependence between these two variables.

In our study we did not find any correlation between serum tIgE and vitDs level (rho = -0.126, p = 0.126). However, there is a trend that the mean value of vitDs level decreased with age, while the serum concentration of total IgE increased with age. When we investigated separate correlations between serum tIgE and vitDs in individual allergic diseases, we noticed a significant negative correlation between them in children who had nettle rash (p = 0.000) and asthma comorbidity with atopic dermatitis (p = 0.000).

We found statistically significant differences in serum tIgE between boys and girls (p = 0.004). The mean total serum IgE in boys was higher (383.35 ± 519.91 IU/ml) than in girls (256.23 ± 546.11 IU/ml). Simultaneously, there is a statistically significant difference in serum 25(OH)D level between boys and girls (p = 0.020) so they maintain the same parity (21.98 ± 8.9 *vs*. 18.47 ± 6.9 ng/ml).

There was a statistically significant difference between child's age and positive/negative SPT (p = 0.004). The mean age of children who had positive SPT was 7.5 ± 3.8 years and of children who had negative SPT it was 5.1 ± 3.1 years. Also, we found a statistically significant difference between a child's age and increased sIgE ≥ 3 class (p = 0.004) (7.8 ± 3.3 *vs*. 6 ± 4.2 years).

CONCLUSION

We found a significant dependence of positive SPT and high serum sIgE ≥ 3 class to certain allergens from the low serum 25(OH)D level (insufficiency or deficiency), which means that vitD contributes to reactivity to a certain allergen. We noted the correlation of increased tendency to allergies and, simultaneously, low level of vitamin D with a child's age. Also, we confirmed the dependence of comorbidity asthma from hypovitaminosis D. We noticed a significant dependence of serum tIgE from the vitDs in children who had nettle rash or with comorbidity of asthma and atopic dermatitis. We did not find the correlation between serum tIgE level and vitD level for the whole group of participants. Children with hypovitaminosis D exhibited a more pronounced tendency to one or more allergic diseases.

Our findings suggest that the vitDs level could be determined synchronously with known markers of allergic status with the goal of precisely determining the child's allergic status. Perhaps correction of hypovitaminosis D would affect the decrease in the prevalence of allergic diseases. In order to gain full insight into the allergic status of children in the future, we need to conduct further investigations of the relationship between serum 25(OH)D level, tIgE level, sIgE ≥ 3 class, and SPT.

Conflict of interest: None declared.

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Зависност маркера алергијског статуса од концентрације витамина Д у серуму

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САЖЕТАК

Увод/Циљ Недавне студије су доказале везу између ниске серумске концентрације витамина Д и пораста преваленце алергијских болести.

Циљ овог рада је да покаже да ли постоји зависност маркера алергијског статуса: кожног (*prick*) алерготеста (КАТ), концентрације укупног имуноглобулина Е (уИгЕ) и концентрације ИгЕ специфичног за алерген (класа сИгЕ ≥ 3) у серуму од серумске концентрације 25(*OH*)*D* (витДс) код деце оболеле од алергијске болести.

Методе У ову студију је било укључено 150 деце са алергијским болестима. Процењени су, истовремено, ВитДс, уИгЕ, КАТ и сИгЕ ≥ 3 класе на инхалаторне и нутритивне алергене.

Резултати Утврдили смо негативну корелацију између нивоа витД и старосних група и статистички значајну позитивну корелацију између витДс и, с друге стране, уИгЕ, сИгЕ ≥ 3 класе на кокошје жуманце и беланце. Статистички значајна позитивна корелација утврђена је између витДс и КАТ на кућну грињу и негативна корелација између уИгЕ и КАТ на кућну грињу, као и између витДс и сИгЕ ≥ 3 класе на гљивице *Cladosporium* и *Alternaria*. Потврдили смо зависност копривњаче и коморбидитетне астме од инсуфицијенције витамина Д и дефицијенције витамина Д. Нисмо нашли зависност уИгЕ од витДс за цео узорак.

Закључак Да би смо добили адекватан увид у алергијски статус деце, морамо уважити плеотропне ефекте витамина Д, сходно чему предлажемо да се, убудуће, одређује витДс синхроно са познатим маркерима алергијског статуса.

Кључне речи: имуноглобулин Е; витамин Д; деца; алерген

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Early and midterm results after surgical repair of anomalous origin of the left coronary artery from the pulmonary artery

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SUMMARY

Introduction/Objective The anomalous origin of the left coronary artery from the pulmonary artery (ALCAPA) is rare congenital disease, which causes myocardial ischemia and subsequent heart failure in infants. The aim is early and mid-term follow up evaluation of the heart function after surgical repair of ALCAPA.

Methods Investigation was retrospective and included medical records of the ALCAPA patients treated surgically, between 2009 and 2017, at the tertiary referent heart center.

Results Five patients (four girls) with coronary anomaly were included in the study. All patients had significantly increased left ventricular end diastolic diameter (z-score 6.6 ± 2.43) and left atria size (z-score 3.09 ± 0.37), along with decreased systolic function (ejection fraction 34.8 ± 7.4% and fractional shortening 15.5 ± 3.4%). The surgery was performed on average at the age of 8.2 ± 7.8 months. Operative treatment was associated with early improvement in echocardiographic parameters (except the size of the left atria). Patients were followed for 4.5 ± 2.6 years. Improvement in echocardiographic parameters was age-related. Patients under four months had recovery early after surgery, those treated at 5.5–6 months of age had normalization after 12 months, and patient who was recognized in the second year of life had late recovery (after ≥ 24 months).

Conclusion Operative treatment in the first 3–4 months of life is related with the most favorable prognosis and rapid normalization of the echocardiographic parameters.

Keywords: ALCAPA; cardiomyopathy; echocardiography

INTRODUCTION

The anomalous origin of the left coronary artery from the pulmonary artery (ALCAPA), also known as Bland-White-Garland syndrome, is a rare congenital disorder with prevalence of 0.25–0.5% and produces postnatal myocardial ischemia with the clinical presentation of the heart failure and mitral regurgitation [1, 2]. The symptoms occur after 6–8 weeks of life due to decrease in pulmonary vascular resistance and coronary steal phenomenon [1, 2, 3]. Ischemia is initially transient and the symptoms present only in periods of increased oxygen demand (e.g. feeding and crying). However, persistent myocardial ischemia induces microenvironment changes and subsequent congestive heart failure [2]. Historically, ALCAPA caused 90% deaths in infants. Advanced surgical methods have significantly reduced mortality rate (0–17%) [1, 3]. The aim of our investigation was evaluation of the heart function and reverse remodeling during early and mid-term follow-up after surgical repair.

METHODS

This study was conducted at the Dr Vukan Čupić Mother and Child Health Care Institute of Serbia and it reflected a period of eight years (2009–2017). We performed a retrospective analysis of medical records. Five patients were included in the study, and no patients were excluded.

The diagnosis was established by echocardiography and confirmed in two patients using cardiac catheterization after symptoms (sweating, feeding, and thriving difficulties and recidivism of bronchiolitis), specific ECG changes (anterolateral wall ischemia) and biochemical parameters appeared. The echocardiographic finding included abnormal movements of the anterolateral wall, left ventricular dilatation, systolic dysfunction, mitral regurgitation, and pathological origin of left coronary artery along with reverse blood flow in the pulmonary artery. Congestive heart failure was treated with diuretics (furosemide, spironolactone), ACE inhibitors (captopril) and cardiotonics (milrinone, dopamine, dobutamine), along with aspirin and fraxiparine during the average period

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of two weeks after diagnosis was established. All patients were treated surgically. The anomalous coronary artery is excised from pulmonary artery (PA) with button of PA wall and reimplanted into the left sinus of Valsalva without tension and torsion. For myocardial protection, crystalloid cardioplegic solution was used. The associated mitral valve surgery was not preformed.

Follow-up parameters were obtained every six months. Left ventricular end-diastolic diameter (EDD), end-systolic diameter (ESD), ejection fraction (EF), fractional shortening (FS), left atria (LA) and aortic dimensions, and mitral regurgitation were observed by M-mode, 2-D, Doppler echocardiography and z-scores estimations [4]. Mitral regurgitation was classified as mild (1+), moderate (2+), and severe (3+) [5].

Data are presented as percentages, mean, and standard deviation (SD). The analytic strategy included paired t-test for group comparisons. Relation between numeric variables is presented graphically by means and 95% confidence intervals. Analyses were performed in SPSS 23.0 for Windows, and 0.05 level defined statistically significant result. The study was approved by the institutional Committee on Ethics.

Table 1. Patient's characteristics and initial preoperative echocardiographic parameters

Age at the time of surgery (months)	8.2 ± 7.8
Body weight (kg)	6.5 ± 1.7
Girls	4/5
Left ventricle EDD (mm)	42.2 ± 7.56
Left ventricle all (z-score) Age 3.5–4 months Age 5.5–6 months Age 22 months	6.60 ± 2.43 7.53 ± 4.19 5.52 ± 1.37 6.89
All (%) Age 3.5–4 months Age 5.5–6 months Age 22 months	34.8 ± 7.4 32 ± 11.31 39 ± 5.66 32
FS (%)	15.5 ± 3.4
LA (mm)	22.5 ± 1.3
LA (z-score)	3.09 ± 0.37
LA:Ao	1.9 ± 0.4
Mitral regurgitation (MR)	Moderate (2/5), severe (3/5)

EDD – end-diastolic diameter; EF – ejection fraction; FS – fractional shortening; LA – left atria; Ao – aorta

RESULTS

Five patients were included in the study, and their preoperative characteristics are presented in Table 1. Four patients had surgery in the first year of life (average 4.3 ± 1.2 months), while one patient had a late diagnosis and intervention at 22 months of age. All our patients underwent surgery within 15 days after being diagnosed.

Trend toward statistically significant improvement in echocardiographic parameters was revealed immediate after surgery. Namely, left ventricular EDD decreased in size (34.8 ± 9.9 mm; z-score 3.48 ± 2.18; p = 0.053), and both EF (52.8 ± 14.6%; p = 0.069) and FS (27.0 ± 7.2%; p = 0.063) increased in early postsurgical period (Table 2).

Echocardiographic follow-up (FU) was 4.5 ± 2.6 years (up to 8.1 years) after surgery. During follow-up, significant improvement in cardiac remodeling and contractility was registered (Table 2). Statistically significant improvement in left ventricular EDD z-score was demonstrated six months after surgical treatment (p = 0.02). However, complete myocardial recovery with significant improvement and normalization in both EF (64 ± 15.3, p = 0.02) and FS (36 ± 6.2, p = 0.01) was revealed after 12 and 18 months of follow-up, respectively. Normalization rate in cardiac remodeling and systolic function was related to age at the time of surgery (Figure 1). Patients who had surgery at the age of 3.5–4 months had immediate recovery of ejection fraction (> 60%), and those who were treated at the age of 5.5–6 months had normalization in EF after 12 months. However, one patient who was recognized at the age of 22 months had a late EF recovery (after ≥ 24 months of FU). Similarly, normalization in left ventricular EDD was better in cases with early surgery. Nevertheless, one patient who had surgery at 3.5 months of age had a slightly prolonged EDD normalization regarding myocardial necrosis/infarction and serious dilation (EDD z-score 10.5) at the time of diagnosis. Further improvement

Table 2. Echocardiographic parameters after operative treatment

Parameters	After surgery	6 months FU	12 months FU	18 months FU	≥ 24 months FU
Body weight (kg)	6.6 ± 1.7	9.6 ± 1.7	10.3 ± 1.6	11.5 ± 1.1	19.6 ± 8.2
LVEDD (mm)	34.8 ± 9.9	35.3 ± 9.1	34.5 ± 5.2	34.0 ± 3.7	37.3 ± 4.9
LVEDD (z-score)	3.48 ± 2.19 (p = 0.053)	2.22 ± 2.28* (p = 0.02)	1.91 ± 1.05* (p = 0.009)	1.35 ± 0.97* (p = 0.005)	0.65 ± 0.17* (p = 0.013)
EF (%)	52.8 ± 14.6 (p = 0.069)	56.0 ± 13.8 (p = 0.05)	64.0 ± 15.3* (p = 0.02)	67.3 ± 9.2* (p = 0.007)	66.7 ± 6.7* (p < 0.001)
FS (%)	27 ± 7.2 (p = 0.063)	27.5 ± 7.9 (p = 0.076)	26.0 ± 11.3 (p = 0.40)	36.0 ± 6.2* (p = 0.01)	36.8 ± 3.4* (p = 0.013)
LA (mm)	21.8 ± 4.8	21.0 ± 5.0	24.5 ± 2.1	24.3 ± 2.8	23.9 ± 2.2
LA (z-score)	2.72 ± 1.47 (p = 0.633)	1.89 ± 1.57 NA	2.59 ± 0.94 (p = 0.76)	2.21 ± 0.63 (p = 0.64)	1.47 ± 0.50* (p = 0.019)
LA:Ao	1.6 ± 0.3	1.9 ± 0.4	1.6 ± 0.2	1.6 ± 2	1.4 ± 0.2
MR: mild/moderate/severe	0/2/3	0/2/3	1/2/2	1/2/2**	

LVEDD – left ventricle end-diastolic diameter; EF – ejection fraction; FS – fractional shortening; LA – left atria; Ao – aorta; NA – not available;
*statistically significant improvement regarding finding before surgery;
**one patient had mitral valve surgery

in echocardiographic parameters persisted during long term follow-up (≥ 24 months) in all patients, and included normalization of the LA diameter (z-score 1.47 ± 0.50; p = 0.019). There were no death outcomes in both early and late period after surgery.

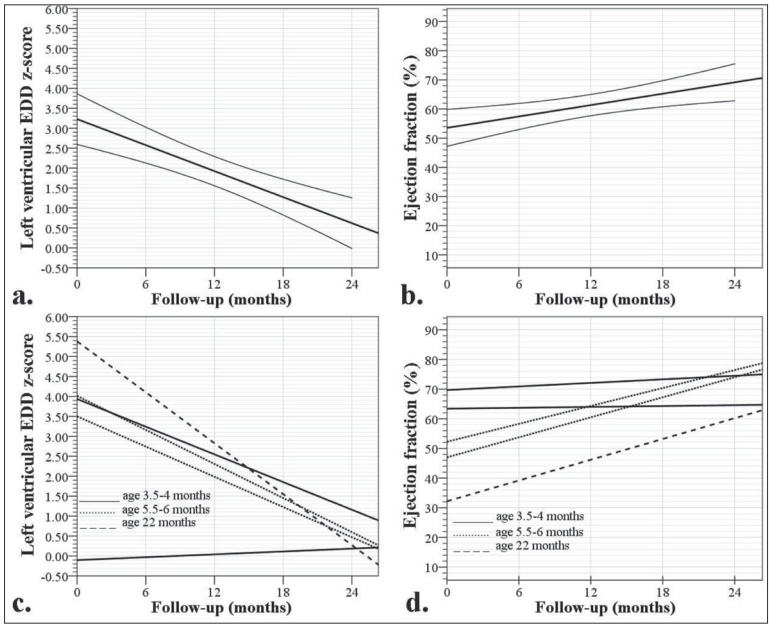

Figure 1. Recovery of the left ventricular end-diastolic diameter (EDD) and ejection fraction after surgical treatment (a. and b. lines represent means and 95% confidence intervals); graphs do not include preoperative values of EDD and ejection fraction

DISCUSSION

The anomalous origin of the left coronary artery from the pulmonary artery causes heart failure in the early infancy. However, clinical symptoms are rare before 6–8 weeks of life resulting from appropriate coronary blood flow due to high pressure in pulmonary circulation. After the second month of life, hemodynamic changes are related to coronary steal phenomena, consequent ischemia and impaired left ventricular function [2]. In our study, patients underwent surgical treatment at 8.2 ± 7.8 months of age and diagnose was established 15 days earlier. Early diagnosis and surgical treatment of ALCAPA are crucial for favorable prognosis, and the management of the mitral regurgitation in the same procedure remains controversial (1–3). We confirmed that the patients' age at surgery influenced the time needed for recovery. Normalization of the cardiac function was reached in first days after surgery in patients who were treated in the first four months of life; in those who had operation at 5–6 months, normalization was present after one year; and in patient with late surgery (second year of life) recovery was demonstrated after 1.5–2 years. In our investigation, mitral valve repair was not preformed.

The differences in recovery could be related to postnatal development of the cardiomyocytes. Infantile cardiomyocytes are small and round cells with 30% contractile proteins and 70% non-contractile mass (membranes, connective tissues, and organelles). In normal conditions, myocardial growth is based on cardiomyocytes enlargement and proliferation, along with cardiogenic/progenitor cells differentiation. Dynamic proliferation changes are revealed during the first year of life [6]. Namely, proliferative capacity in 1–3 months of age is 11 times bigger than at the age of six months, and 27 times at the age of one year [7]. Thereby, better recovery after cardiac surgery (not only for ALCAPA patients) could be expected in younger infants. Expected number of terminally differentiated cardiomyocytes in older surgical age groups is smaller, and those patients are much more sensitive to heart failure development during adulthood [6, 7].

In addition, the duration of myocardial hibernation may influences on postnatal development in patients with ALCAPA. Namely, normal circulation and microenvironment is needed for sufficient myocardial maturation. Mollova et al. [8] showed that manipulation with endogenous mechanisms could change myocardial cell differentiation in infancy. Similarly, survival, integration, proliferation, and differentiation of the STEM cells, and fetal cardiomyocytes (with viability area increment that improves systolic function) is possible if extracellular matrix microenvironment is optimal [9]. In addition, cyanosis and chronic hypoxia in rat model increases cardiac mass in first four weeks of life mainly due to myocardial proliferation [9]. Consequently, hibernated myocardium should be rescued from irreversible ischemia as soon as possible [1, 2, 3, 10, 11].

The optimal change in the microenvironment after surgical treatment of ALCAPA could stimulate postponed proliferation and maturation, which cause significant improvement of both systolic and diastolic cardiac function in our patients (Figure 1). Additionally, almost complete resolution of the myocardial scar has been showed after surgery [10]. Our results showed that normalization in left ventricular remodeling (EDD z-score < 2) and systolic function (EF > 60%) could be expected 12 months after surgery, with additional normalization in the left atria size after two years of follow-up. Weigand et al. [12] analyzed prognosis after surgery of ALCAPA and patients were divided in two groups (infant and non-infant). They have showed that the preoperative EDD was the only independent factor for time to normalization of LV function. However, infant group (up to 12 months) was not subdivided in that study [13]. Finally, patients with ALCAPA can deploy irreversible changes and myocardial infarction. In our patients, range of the myocardial injury before surgery (presented as impaired echocardiographic finding) was not significantly different, and age related capability for myocardial recovering was essential for prognosis.

In spite of appropriate recovery of the LV function after surgical treatment in majority of patients, complications as persistent mitral regurgitation, congestive heart failure, and coronary stenosis are noticed and lifelong echocardiographic follow up is necessary [2, 3, 9]. During FU period,

one of our patients had mitral valve repair. Pro-BNP analysis can help in functional assessment of patients with heart failure. Reoperation or heart transplantation are indicated in cases with unsuccessful either surgical or conservative treatment [2, 6].

Our clinical investigation can establish hypothesis about age-related difference in myocardial recovery based on cardiomyocytes proliferation in patients with ALCAPA. Further basic studies in these patients should be conducted, with aim to establish the relation between age at the time of surgical treatment and cardiac recovery.

CONCLUSION

ALCAPA is one of the most important causes of heart failure in infants. Normalization of the left ventricular systolic and diastolic function is expected 12 months after surgery. In our opinion, diagnosis and operative treatment done in the first months of life are related to favorable prognosis and fast normalization of echocardiographic parameters.

Conflict of interest: None declared.

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Рани и средњорочни резултати хируршког лечења аномалног исходишта леве коронарне артерије из плућне артерије

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САЖЕТАК

Увод/Циљ Аномално исходиште леве коронарне артерије из плућне артерије (*ALCAPA*) ретка је конгенитална аномалија, која проузрокује исхемију миокарда и срчану инсуфицијенцију код одојчади.

Циљ студије је евалуација срчане функције током раног и средњег периода праћења оперисаних болесника.

Методе Студија је била ретроспективна и анализирала је медицинску документацију болесника којима је оперативно лечена *ALCAPA* у периоду од 2009. до 2017. у терцијарној установи.

Резултати Пет болесника (четири девојчице) укључено је у студију. Сви болесници су имали значајно повишен енддијастолни дијаметар леве коморе (*z*-скор 6,6 ± 2,43) и леве преткоморе (*z*-скор 3,09 ± 0,37), уз ослабљену систолну функцију (ејекциона фракција 34,8 ± 7,4% и фракција скраћења 15,5 ± 3,4%). Просечна старост болесника на операцији је била 8,2 ± 7,8 месеци. Операција је повезана са раним побољшањем ехокардиографских параметара (осим дијаметра леве преткоморе). Параметри су праћени 4,5 ± 2,6 година. Побољшање ехокардиографских параметара је било повезано са старошћу болесника. Болесници који су били оперисани ≤ 4 месеца имали су рани опоравак после хирургије, они који су третирани у узрасту 5,5–6 месеци имали су нормализацију после 12 месеци од операције, а болесник који је препознат у другој години живота имао је касни опоравак (после ≥ 24 месеца).

Закључак Оперативно лечење у прва 3–4 месеца живота је повезано са најповољнијом прогнозом и најбржом нормализацијом ехокардиографских параметара.

Кључне речи: *ALCAPA*; кардиомиопатија; ехокардиографија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Surgical treatment for breast tumors in children

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SUMMARY

Introduction/Objective Fibroadenoma, often called "breast mice tumors" due to their mobility, are the most common breast tumors in pediatric population. Considering that some tumors have a potential for rapid growth, breast tissue damage, and that an ideal diagnostic tool has yet to be found, complete mass extirpation might be the treatment of choice. The aim of the study was to present our clinical experience in treating children with breast masses.

Methods A retrospective review (2011–2018) of patients treated for breast tumors at the Institute for Child and Youth Health Care of Vojvodina in Novi Sad was conducted.

Results In this study 29 girls (mean age 15.8 ± 1.8) were included. The majority of masses were located in the upper outer (27.6%) or lower inner (24.1%) breast quadrant. The mean mass diameter was 39.7 mm. It has been observed that the mean mass diameter in the group of girls with positive family history for breast diseases was significantly lower ($p < 0.05$) than in those with negative family history (27.5 *vs*. 43.2 mm). There were no proven malignant tumors and all tumors have been completely extirpated. The mean postoperative stay was 1.5 ± 1.02 days.

Conclusion An appropriate radical operative technique dependent on mass size and localization is still the "gold standard" for treating breast masses in pediatric patients. Cooperation with experts in the field of oncologic breast surgery enables implementing these operative techniques in clinical practice of pediatric surgeons.

Keywords: breast fibroadenoma; phylloid breast tumor; surgery; children

INTRODUCTION

When a breast mass (lump) is detected, the most feared cause is breast cancer. Fortunately, malignant breast tumors are quite rare in children. When breast tumors are presented in adolescent girls, they commonly include hematological (cutaneous T-cell lymphoma) or metastatic diseases, chest wall malignancies, post radiation breast cancer, sarcoma, primary breast cancer (invasive secretory carcinoma), and hereditary breast cancer (Cowden syndrome) [1, 2]. However, in the majority of patients the first causes of the lump to consider are benign tumors such as fibroadenoma [3]. The mass is most commonly found in the upper outer breast quadrant [4]. In 85–90% of cases, breast fibroadenoma is unilateral, but bilateral presentations as well as tumor multiplicity may be observed as well [5].

Clinically, breast fibroadenoma may be completely asymptomatic. However, during physical examination they usually present as smaller or bigger rubbery, painless breast masses, and freely mobile under the skin. Because of their mobility, these tumors are often called "breast mice tumors" [5].

Histologically, fibroadenoma consist of polyclonal epithelial and stromal cells. Therefore are considered to present hyperplastic masses, whose occurrence might be related to an aberrant maturation of the breast tissue [3, 6]. Tumor development is believed to be caused by *MED12* (mediator complex subunit 12) exon 2 somatic mutation [3].

Different radiological modalities may be used for assessment of breast diseases. MRI (magnetic resonance imaging) provides precise images without exposing patients to radiation, but its efficacy and accuracy in children with breast tumors has not been established yet. The modality of choice for the majority of pediatric patients may be ultrasound (US) [2, 7]. This diagnostic tool is capable of providing multiple images during a follow-up period, without a risk of causing any harm to a patient. In addition, younger patients are not candidates for mammography, due to a predominantly glandular breast configuration and known dangerous effects that radiation exposure may cause. Fibroadenoma observed under US presents as oval or round well-circumscribed hypoechoic masses with variable posterior acoustic alteration, and heterogeneous or homogeneous internal texture. These tumors are either avascular or with mildly increased blood flow on Color Doppler [7].

There are two subtypes of breast fibroadenoma: simple and complex. Tumor might be considered complex if it contains at least one of the following characteristics: epithelial calcifications, apocrine metaplasia, sclerosing adenosis, or cysts larger than three millimeters. Precise

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guidelines for making ultrasonographic distinction between simple and complex ones have not been established yet. This is clinically important, knowing that their behavior is quite different. Simple breast fibroadenoma is not associated with increased risk of developing breast cancer. Also, these tumors may remit spontaneously up to 10%. Therefore, conservative management may be feasible in many cases [4, 8]. On the other hand, patients with complex fibroadenoma are believed to have 3.1 times higher chances for developing breast cancer compared to general population [9].

Masses that are solid, non-mobile, enlarging, tender, fixed to overlying skin or nipple-areolar complex, and/or associated with axillary or supraclavicular lymphadenopathy should be suspected for malignancy [4]. A risk factor for developing breast cancer in young patients may be radiation exposure during breast development. When it occurs, breast cancer in these patients tends to be of the secretory variety, with less metastatic potential [10].

Another type of tumor, which might clinically appear as fibroadenoma is phylloid breast tumor. It consists of the same cell types as fibroadenoma, but with stromal (connective) tissue predominance, as well as cellular atypia in some forms [11].

Clinically similar to these tumors, but often presented with serous or sero-sanguinous nipple discharge is intraductal papilloma. This tumor represents a benign proliferation of ductal epithelium. On US it can be seen as an echogenic intraductal mass with internal vascularization on Doppler imaging. In some cases, ductal dilatation may be observed as well [12].

It is sometimes hard to choose a proper therapeutic management for breast masses in young patients. Firstly, the risk of a malignant disease must be estimated. However, only 0.02% of surgically removed pediatric breast masses are proven malignant [10]. There are some factors in patient's history, which may indicate if observed mass requires specific attention, in order to exclude potential malignancy. The most important one is family history of breast cancer [13–16]. Patients with a clinical history of chest radiation (for example for childhood Hodgkin's disease) should also be carefully examined, due to the relatively high rates of breast cancer [17].

Considering specific (predominantly glandular) breast structure in adolescent girls, iatrogenic damage to the developing breast tissue must be avoided as much as possible. In addition, generalized anxiety over having a breast lump, as well as cosmetic changes that may occur might be significant factors when determining appropriate management for young patients [4, 18].

Potential malignancy of tumor mass might be determined using less invasive techniques such as fine needle aspiration (FNA). It has recently been proven that this technique may not help making a precise differentiation between a fibroadenoma and phyllodes tumor, therefore it is not required [9, 19].

Considering that some of these tumors have a potential for rapid growth, and breast glandular tissue damage, as well as that an ideal diagnostic tool has not been found yet, complete mass extirpation might be the treatment of choice [19]. Breast masses in children and adolescents require particular attention, both before and after surgical intervention. Of course, much more attention is paid if tumor is histologically proven complex fibroadenoma or breast cancer. On the other hand, some authors advocate that in benign diseases, such as simplex fibroadenoma, after tumor extirpation follow-up is not necessary any more [9].

The aim of the study was to present our clinical experience in treating children with breast masses, as well as to compare treatment options and outcomes with results recently published in studies worldwide.

METHODS

This retrospective review of patients, treated for breast tumors between 2011 and 2018, was done in accord with standards of the institutional Committee on Ethics. All patients were diagnosed and treated at the Clinic of Pediatric Surgery, Institute for Child and Youth Health Care of Vojvodina, in Novi Sad. Diagnoses were made by taking history, performing physical and expert US examinations (routinely performed at the Diagnostic Imaging Center, Oncology Institute of Vojvodina, Sremska Kamenica). All extirpated masses were analyzed histologically at the Oncology Institute of Vojvodina in Sremska Kamenica, as well.

Recorded data were analyzed using Microsoft Office Excel 2007 and IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Data were described using frequencies, percentages, means, standard deviations, and bivariant correlations where appropriate. Between-group differences were analyzed using the independent-samples t-test. Calculated differences lower than significance levels of 0.05 were considered relevant.

RESULTS

In this retrospective chart review, 29 girls aged between nine and 18 years (mean age 15.8 ± 1.8) were analyzed (Table 1).

The majority of masses were located in the upper outer (27.6%) or lower inner (24.1%) breast quadrant. In 13.8% of participants, the tumor was in lower outer quadrant. Masses in both upper or in both lower quadrants were observed in 6.9% of patients each (Figure 1).

The mean mass diameter was 39.7 mm (range 10–70 mm) (Table 2). In 10 patients, tumors were equal to or larger than 50 mm in diameter.

The breast mass was painful in 34.5% of participants (Figure 2). There was no difference ($p > 0.05$) in diameter between painful and painless breast masses (Tables 3 and 4). One girl with a painful mass was diagnosed with phylloid breast tumor, while in others fibroadenoma was proven.

There were no verified malignant tumors. Of all patients analyzed, just one was histologically proven with phylloid breast tumor, while all the others had breast fibroadenoma.

In majority of patients (79.3%), family history was negative for breast diseases (Figure 3). It has been observed

Table 1. The age of patients analyzed in the study

Parameters	n	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Age	29	9	18	**15.8**	1.8	-2.1	0.4	6.8	0.8

Table 2. The breast mass size in our patients

Parameters	n	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error		Statistic
mass diameter (mm)	27	10	70	**39.7**	16	-0.5	0.5	-0.8	0.9

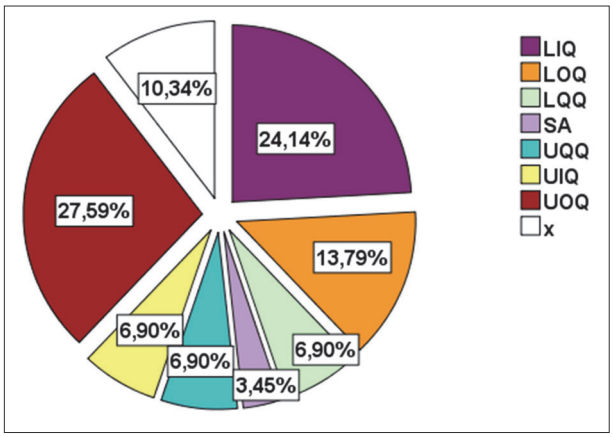

Figure 1. Localization of the mass in the breast

LIQ – lower inner quadrant; LOQ – lower outer quadrant; LQQ – both lower quadrants; SA – subareolar; UQQ – both upper quadrants; UIQ – upper inner quadrant; UOQ – upper outer quadrant; x – missing data

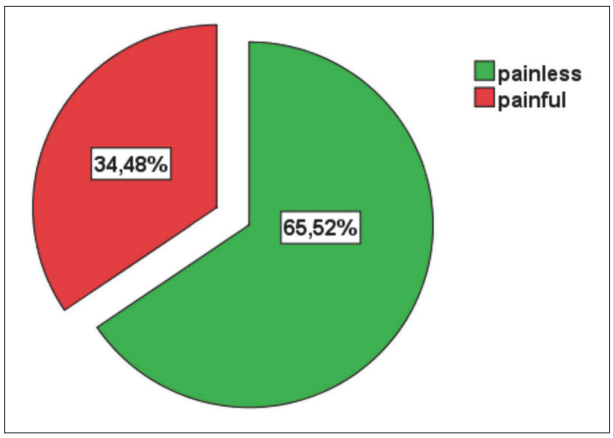

Figure 2. Pain presentation in our patients

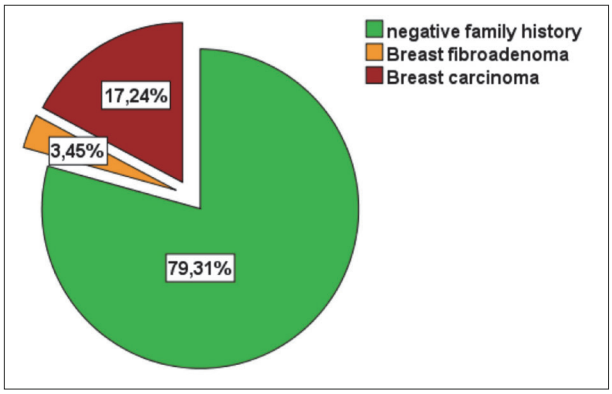

Figure 3. Family history of breast tumors

Table 3. The mean mass diameter at presentation in patients with pain and in those without it

Parameters	Pain	n	Mean	Std. Deviation	Std. Error Mean
Mass diameter (mm)	Painless	17	**39.7**	15.8	3.8
	Painful	10	**39.7**	17.05	5.4

that the mean mass diameter in the group of girls with positive family history for breast diseases was significantly lower ($p < 0.05$) than in those with negative family history (27.5 mm vs. 43.2 mm) (Table 5 and 6).

Tumors have been completely surgically extirpated in all patients. The mean postoperative hospital stay was 1.5 ± 1.02 days (range 1–5) (Table 7).

During the postoperative follow-up period (seven months – seven years) only one recurrent tumor was observed, 10 months after the initial operation of phylloid tumor. Histology confirmed diagnosis of fibroadenoma.

DISCUSSION

Fibroadenoma is quite common during reproductive period, approximately 25% of women, while incidence in adolescent girls is approximately 2% [5, 18]. These tumors may occur at any age, but the peak incidence is reported in the second and third decade [18, 20]. In our study, the mean age of patients was 15.8 years old (range 9–18).

Table 4. Comparison of mass diameters in patients with painful and painless breast masses

Parameters		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mass diameter (mm)	Equal variances assumed	0.2	0.7	0.001	25	**0.99**	0.006	6.5	-13.3	13.4
	Equal variances not assumed			0.001	17.8	0.99	0.006	6.6	-13.9	13.9

Table 5. The mean mass diameter in patients with positive family history of breast diseases and in those without it

Parameters	Family history	n	Mean	Std. deviation	Std. error mean
Mass diameter (mm)	Negative	21	**43.2**	14.6	3.2
	Positive	6	**27.5**	15.4	6.3

Table 6. Difference in the mean mass diameter in patients with positive family history of breast diseases and in those without it

Parameters		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% confidence interval of the difference	
									Lower	Upper
Mass diameter (mm)	Equal variances assumed	0.01	0.9	2.3	25	**0.03**	15.7	6.8	1.6	29.8
	Equal variances not assumed			2.2	7.8	0.06	15.7	7.06	-0.7	32.04

Table 7. Days spent in hospital after the surgery

Parameters	n	Minimum	Maximum	Mean	Std. deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. error	Statistic	Std. error
Postoperative stay (days)	29	1	5	1.5	1.02	2.2	0.4	4.6	0.8

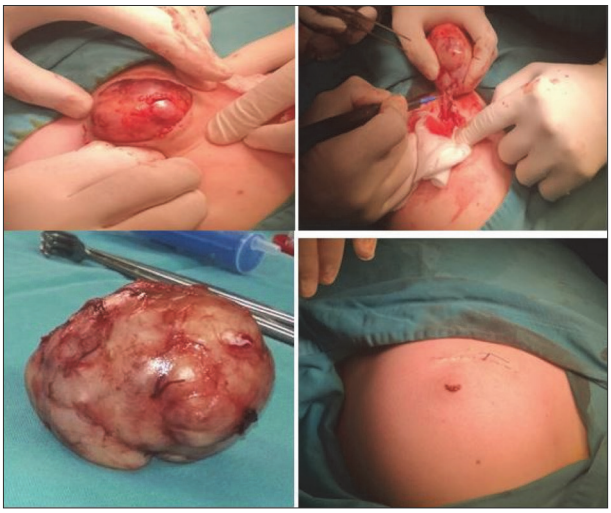

Figure 4. Giant breast fibroadenoma extirpation

Taking into account that these tumors are estrogen dependent, their extremely rare occurrence in prepubertal girls, as well as their increased growth in periods when estrogen levels are high, such as in puberty, pregnancy or lactation may be explained [8]. In pubertal girls, estrogen provides growth of lactiferous ducts, whereas progesterone stimulates differentiation of lobules and alveoli. Breast development may be clinically stratified into five Tanner stages. These clinical stages have their own specific US appearance, and must not be mistaken for breast tumors [2].

Various normal anatomical structures might clinically appear as breast tumors, such as normal lymph nodes, breast bud asymmetry, prominent osseous structures (chest wall deformities or Poland syndrome). Physiological breast development might also be disturbed. As a result, ectopic breast tissue may be found, usually along the so called "milk line". If breast tissue fails to involute during male development, in pubertal boys' gynecomastia may occur. In literature, it is reported in up to two thirds of boys aged 10–13. In our study, there were no boys analyzed.

Non-neoplastic breast lesions are relatively common in children, and these include breast hematomas, cysts, galactoceles, and abscesses [2].

Despite all these possible etiological factors, the most important causes of breast masses to consider are neoplastic lesions, benign and malignant. All but one patient (phylloid breast tumor) in our study had benign breast diseases (fibroadenoma).

In current literature, the most common localization of breast tumors is reported to be in the upper outer quadrant of the breast, probably due to the highest concentration of glandular tissue in this area [4]. In our study, besides previously mentioned location, we found a large number of masses located in the lower inner breast quadrant.

Considering that breast malignancies are quite uncommon in children, as well as possibility of potential damage to developing breast tissue, we emphasize "first do no harm" in both diagnostic and therapeutic approaches [2]. For this reason, we have cooperated with our colleagues from the Institute of Oncology in Sremska Kamenica, whose experience in treating adult patients with breast pathology is great. Besides that, precise and gentle surgical technique enabled us to preserve noble glandular breast tissue and leave it undamaged.

US is the modality of choice for diagnosing breast diseases in majority of pediatric patients, and BI-RADS (Breast Imaging Reporting and Data System) lexicon is the gold standard for describing and stratifying these masses. Using this lexicon, seven different groups of masses (labeled 0–6) can be described. Masses categorized as BI-RADS 4 or above are suspicious for malignancy [2]. Characteristics considered as possible indicators that one mass is not a fibroadenoma (but e.g. phylloid tumor) include maximum diameter greater than 4 cm, irregular borders, and heterogeneous echogenicity with intralesional cystic areas [21].

The mean tumor diameter in our study was 39.7 mm (range 10–70 mm). Fibroadenoma size usually ranges from a few millimeters to a few centimeters. Rarely, these tumors may cause enormous and/or rapid breast enlargement. If fibroadenoma larger than 50 mm in diameter or replaces at least 80% of the breast, it is named giant fibroadenoma [19]. Giant fibroadenoma that present in adolescent patients are called juvenile fibroadenoma [9]. It occurs in approximately 4% of all adolescents with breast mass and was present in 10 of our patients (37%) [21, 22, 23]. For juvenile fibroadenoma, which are symptomatic or rapidly growing, prompt surgical excision is appropriate. When complete excision of this tumor is performed, specific reconstructive techniques might be required [4]. Although one of the members of our surgical team was a plastic surgeon, none of our patients needed reconstructive breast surgery.

Fibroadenoma is known to present as smaller or bigger "rubbery", painless masses, freely mobile under the skin [2, 4, 5]. Despite that, 34.5% of our patients reported that the mass was painful. Our patient who was diagnosed with phylloid breast tumor was complaining of pain. This finding requires further investigation, in order to determine factors that might affect variances in clinical presentation of different types of breast tumors.

Phylloid breast tumor (cystosarcoma phyllodes) is quite a rare neoplasm, accounting for less than 0.5% of all breast tumors in all age groups. High local recurrence rate as well as sometimes-malignant potential signifies its importance [11, 24, 25]. Phylloid tumors of the breast can be usually classified as benign, borderline, or malignant [11]. In literature, benign forms have local recurrence rate in approximately 20% of cases; borderline in 14–25%, while malignant forms approximately 14–40%. Malignant phylloid breast tumors might cause metastatic disease in approximately 9–27%. Metastatic potential of borderline phylloid tumor is not certain, but few cases have been reported [11].This is the reason why all extirpated masses were histologically analyzed at the facility which is highly experienced in this pathology.

It is clinically hard to determine a clear difference between breast fibroadenoma and phylloid tumor. Masses that grow fast, as well as large masses are under suspicion of phylloid tumors. There are authors who suggest that every breast mass larger than 30 mm should be considered as phylloid tumor [21]. Larger tumor size and positive resection margin are well known to be risk factors for local tumor recurrence. Recurrence tumor can be lower or the same grade as the initial one, but in majority of cases, tumors with a more aggressive growth and enhanced malignancy are found on recurrence [26, 27, 28]. In our study, a girl that had been operated for phylloid tumor had a recurrent tumor 10 months after thr initial surgery. Histology tests confirmed fibroadenoma.

In the majority of girls included in the study, family history was negative for breast diseases. One girl (3,4%) had a positive family history for breast fibroadenoma, whereas another five girls (17.2%) had a breast cancer in their family history, which makes a total of six patients (20.6%) with known breast diseases in their families. In literature, positive family history for breast diseases is reported in approximately 8.6–24.4% of patients with breast masses [29].

Several genes, such as *TP53* (tumor suppressor protein 53), *BRCA1* and *BRCA2* (breast cancer genes), *PMS2* (post meiotic segregation increased 2 protein), *NF1* (neurofibromin protein 1 gene), *APC* (adenomatous polyposis coli gene), *RB1* (retinoblastoma associated protein 1) and *AML1* (acute myeloid leukemia protein 1 gene) are proven to be associated with breast cancer occurrence in some families [13–16].

The mean tumor diameter at presentation in group of girls with positive family history for breast diseases was significantly lower than in those with negative family history (difference was approximately 15 mm). Possible explanation might be in increased awareness of breast pathology among girls who have a relative with breast tumor, as well as their justified fear of developing cancer themselves.

Choosing an appropriate management for breast tumors in young patients is a challenge. Some authors advocate that probably benign masses up to 30–40 mm in diameter might be safely followed-up, except when they have tendency of rapid growth or cause significant clinical symptoms. These authors suggest that masses larger than 50 mm must be biopsied [2]. However, biopsy is not as harmless as it might appear, and iatrogenic damage of developing breast tissue has been reported [30]. On the other hand, there are also authors who believe that tumors sized 30 mm should be considered as phylloid breast tumors and surgically treated [21]. There is also important difference whether the breast fibroadenoma is simple or complex. Simple fibroadenoma may resolve spontaneously in up to 10% of cases, therefore may be treated non-operatively [4, 9]. However, in specific cases when these tumors cause anxiety in a patient and/or patient's family, complete extirpation may be appropriate treatment [4, 18, 30].

Operative technique depends on the mass size and location, but complete tumor resection should be the main goal. In cases when phylloid tumor is proven (*ex tempore*) or suspected, R0 resection should be done due to its possible recurrence, rapid growth, and/or metastasis [9, 11]. In all our patients, tumors have been completely surgically removed.

The optimal incision site (circumareolar or inframammary) which minimizes visible scarring is not always possible. When tumor location is further from the areolar border, its resection may be performed through curvilinear or semilunar incisions directly over the mass [4]. When treating giant fibroadenoma in young girls, it is important to make a proper reconstruction of the remaining tissue, in order to keep the breast shape acceptable. This also includes preserving the nipple-areolar complex in proper place, achieving the best possible aesthetic result, as well as enabling lactation in a regenerative period. Healthy breast tissue that had been compressed by expansive tumor lesion might appear diseased, but it has been proven that this tissue has potential of filling the void left after surgical mass excision. This is the main reason why surgical breast reconstruction is rarely required in young patients [19]. If needed, the precise timing for reconstructive surgery has

not been established yet, but some authors recommend performing this operation minimally one year after the initial treatment and after skeletal maturation [18, 30]. None of our patients required breast reconstruction due to a complete *restitutio ad integrum*.

Our study had its limitations, mostly due to a small sample size. Therefore, many mentioned interesting findings should be checked in a larger number of patients. In addition, future studies might consider mechanical trauma as a possible etiological factor for developing breast tumor.

CONCLUSION

In this study, there were no verified malignant tumors. After carefully considering all the treatment options, an appropriate radical operative technique dependent on mass size and localization is still a "gold standard" for treating breast masses in pediatric patients. Cooperation with experts in field of oncologic breast surgery enables implementing these operative techniques in clinical practice of pediatric surgeons.

Conflict of interest: None declared.

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Хируршко лечење тумора дојки у дечјем узрасту

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САЖЕТАК

Увод/Циљ Фиброаденоми, услед своје изразите покретљивости звани и „мишеви дојке", најчешћи су тумори дојке у дечјем узрасту. Узевши у обзир чињенице да тумори дојке могу својим растом да ремете развој њеног нормалног жлезданог ткива и да не постоји идеалан начин за дијагностиковање ових тумора, оперативно лечење са комплетном ресекцијом представља „златни стандард" у терапији.
Циљ ове студије је да прикаже клиничко искуство Клинике за дечју хирургију у Новом Саду у лечењу ове патологије.

Методе Истраживање је конципирано као дескриптивно-ретроспективна студија. Анализиране су болеснице лечене од тумора дојки на Институту за здравствену заштиту деце и омладине Војводине у Новом Саду у периоду 2011–2018. године.

Резултати Студију чини 29 болесница, просечне старости 15,8 ± 1,8 година. Већина тумора је позиционирана у горњем спољашњем (27,6%) и доњем унутрашњем (24,1%) квадранту дојки. Тумори су били просечног пречника 39,7 *mm*. Уочено је како су код болесница са позитивном породичном анамнезом у правцу тумора дојке тумори били мањих димензија у поређењу са оним код болесница са негативном породичном анамнезом (27,5 *mm* у поређењу са 43,2 *mm*). У студији није било малигнитета и код свих болесница начињена је тотална екстирпација тумора. Хоспитализација је у просеку износила један и по дан.

Закључак У зависности од величине и локализације тумора, одговарајућа радикална операција и даље представља „златни стандард" у лечењу тумора дојке код деце. Сарадња се експертима из области онколошке хирургије омогућава да ова патологија постане рутина у раду дечјих хирурга.

Кључне речи: фиброаденоми дојке; филодес тумори; оперативно лечење; деца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pulmonary air leak syndrome in term and late preterm neonates

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SUMMARY

Introduction/Objective Air leak syndrome is more frequent in neonatal period than at any other period of life. Its timely recognition and treatment is a medical emergency. We present results of a tertiary medical center in treatment of air leak syndrome in term and late preterm neonates.

Methods Neonates born between 34th 0/7 and 41st 6/7 gestational weeks (g.w.) who were treated for air leak syndrome in the Neonatal Intensive Care Unit of Mother and Child Health Care Institute, from 2005 to 2015 were included in the study. Antropometric data, perinatal history, type of respiratory support prior to admission, chest radiography, type of pulmonary air leak syndrome and its management, underlying etiology, and final outcome were analyzed.

Results Eighty-seven neonates of an average gestational age 38.1 ± 1.9 g.w. were included in the study. The average birth weight was 3182.5 ± 55.5 g. Fourty-seven (54%) were born by cesarean section and 40 (46%) were born by vaginal delivery. Prior to admission, 62.1% received supplemental oxygen, 4.6% were on nasal continuous positive airway pressure, and 21.8% were on conventional mechanical ventilation. Type of delivery did not significantly affect the appearance of pneumothorax, nor did the type of respiratory support received prior to admission ($p > 0.05$). The majority (93.1%) had pneumothorax, which was unilateral in 79%. The length of mechanical ventilation significantly affected the appearance of pneumothorax ($p = 0.015$). Low Apgar score in the first minute and the presence of pneumopericardium were significant factors predisposing for an unfavorable outcome.

Conclusion Improving mechanical ventilation strategies and decreasing the rate of perinatal asphyxia in term and late preterm neonates could diminish the incidence of pulmonary air leak syndrome in this age group.

Keywords: pneumothorax; newborn; respiratory insufficiency; mechanical ventilation

INTRODUCTION

Pulmonary air leak syndrome (PALS) comprises several different clinical conditions resulting from alveolar over distension and air leakage outside the lungs. It appears more frequently in neonatal period than in any other period of life [1]. This is due to some particularities of respiratory system and its physiology in neonates (poorly compliant lungs, absence of collateral ventilation, highly compliant chest wall, poor respiratory reserve etc.) [2]. Frequency of PALS is determined by gestational age, mode of delivery, underlying lung disease, therapeutic interventions, mechanical respiratory support [3–5].

The aim of the study was to present the frequency, pathogenesis and treatment of PALS in a group of term and late preterm neonates treated in a tertiary medical center. Risk factors, clinical course and outcome of neonates treated for PALS were also analyzed.

METHODS

We present a group of 87 neonates treated for PALS in Neonatal Intensive Care Unit (NICU) of Mother and Child Health Care Institute, Belgrade, from January 2005 to December 2015. All patients were born between 34th 0/7 to 41st 6/7 gestational weeks (g. w.). The following data were analyzed from medical records: gestational age, birth weight, mode of delivery, Apgar score, need for resuscitation and respiratory support after birth, type of PALS (pulmonary interstitial emphysema [PIE], pneumomediastinum, pneumothorax, pneumopericadium), clinical and radiographic findings, accompanying disorders, mechanical ventilation (mode, parameters and duration), treatment of PALS (spontaneous resolution or chest tube drainage) and final outcome. the diagnosis was based on a plain chest radiography. According to the common hospital practice, if thoracic drainage was indicated for treatment of pneumothorax, chest tube was inserted by a pediatric surgeon. Neonates who required mechanical ventilation were ventilated using conventional mechanical ventilation modes. The final outcome was considered favorable if the patient recovered and was discharged home without needing supplemental oxygen. A patient's death was considered an unfavorable outcome.

Categorical variables were identified and reported in percentage. Data were analyzed using SPSS (Kolmogorov–Smirnov χ^2 test for testing

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the normal distribution, Pearson's χ^2 test for testing the association of variables, Student's t-test for testing the difference between groups, univariate logistic regression). A p-value of < 0.05 was considered statistically significant.

The protocol and publication of the results were approved by the Ethics Committee of the Mother and Child Health Institute of Serbia (number 8/10).

RESULTS

During the observed 10-year period, out of 3,484 neonates hospitalized in the NICU, 91 were diagnosed with PALS, which makes 2.6% of all hospitalized neonates. Four neonates were born before 34 g. w. Since the study aimed to analyze term and late preterm neonates, these four patients were excluded and further analysis was based on 87 patients. Patients' demographic characteristics are presented in Table 1. The average gestational age was 38.1 ± 1.9 g. w. The average birth weight was 3,182.5 ± 55.5 g. In the late preterm subgroup, the average birth weight was 2,791 ± 441.9 g, while in the term subgroup it was 3,349 ± 456 g. There is a statistically significant difference in birth weight in these two subgroups of neonates (t = 5.264; p < 0.001).

Mean Apgar score was 7.1 ± 2.4 at the first minute and 7.9 ± 2.1 at the fifth minute. There is a statistically significant difference in Apgar score at the first and fifth minute (t = 6.700; p < 0.001). Apgar score significantly increased at fifth minute.

Forty-seven neonates (54.1%) were born by cesarean section, while 40 (45.9%) were born by vaginal delivery. There is no statistically significant difference in the mode of delivery (p > 0.005).

Type of respiratory support prior to admission to our hospital and type of PALS neonates developed are presented in Table 2.

On admission to the NICU, 77 (88.5%) neonates had pathological auscultatory findings on chest auscultation, 61 (70.1%) had signs of respiratory distress, and 53 (60.9%) had tachypnoea.

Chest radiography was a part of the initial workup and was described by an experienced radiologist as pathologic in 85.1% of neonates, while in 14.9% it was described as normal. Signs of PALS were present in 45 (51.7%) of patients. The distribution of PALS type on admission is presented in Table 2.

In 10 patients there were signs of air leakage in more than one thoracic cavity. The distribution of PALS type in those 10 patients is presented in Table 3. Out of five patients with pneumopericardium, one patient was symptom-free and had only a thin continuous band of lucency encircling the heart with no clinical significance, while four patients had pneumopericardium along with pneumothorax. Three out of five patients with pneumopericardium died.

In patients with pneumothorax, there was a complete pneumothorax in 54 (66.6%) and partial pneumothorax in 27 (33.3%) patients. Pneumothorax was unilateral in 64 patients, most commonly right-sided (60.9%). Patients' distribution in regard to pneumothorax type and its resolution is presented in Table 4.

Spontaneous resolution of pneumothorax was observed in 31% of patients, while 69% needed thoracic drainage. Median thoracic drainage length was four days (range 1–14 days). Median thoracic drainage in patients with unilateral pneumothorax was four days (1–14 days), while it was six days (1–13 days) in patient with bilateral pneumothorax. There is a statistically significant difference in the length of thoracic drainage in regard to the type of pneumothorax (U = 195.5; p = 0.014). After the insertion of thoracic chest

Table 1. Patients' characteristics

Sex		
Male	54 (62.1%)	p > 0.005
Female	33 (37.9)	
Gestational age at birth (g. w.)		
37 0/7–41 6/7	61 (70/1%)	p < 0.005
34 0/7–37 0/7	26 (29.9%)	
Mode of delivery		
Vaginal	40 (46%)	p > 0.005
Cesarean section	47 (54%)	
Average birth weight (g ± SD)		
Term neonates	3,349 ± 456	p < 0.001
Late preterm neonates	2,791 ± 441.9	
Average Apgar score		
1st minute	7.1 ± 2.4	p < 0.001
5th minute	7.9 ± 2.1	
Perinatal asphyxia		
Present	57 (65.5%)	p < 0.005
Absent	30 (34.5%)	

g. w. – gestational weeks

Table 2. Distribution of patients in regard to type of respiratory support and type of pulmonary air leak syndrome

Type of respiratory support	
None	9 (10.3%)
Oxygen	54 (62.1%)
nCPAP	4 (4.6%)
MV	19 (21.8%)
Type of PALS	
PIE	3 (3.4%)
Pneumothorax	81 (93.1%)
Pneumomediastinum	10 (11.5%)
Pneumopericardium	5 (5.7%)

nCPAP – nasal continous possitive airway pressure; MV – mechanical ventilation; PALS – pulmonary air leak syndrome, PIE – pulmonary interstitial emphysema

Table 3. Distribution of pulmonary air leak syndrome type in patients with air leakage in several thoracic cavities

Types	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
PIE	X	X							X	
PM			X	X	X	X			X	
PT	X	X	X	X	X	X	X	X	X	
PP						X	X	X	X	X
Final outcome	+	+	+	+	+	-	-	+	-	+

Pt – patient, PIE – pulmonary interstitial emphysema; PM – pneumomediastinum; PT – pneumothorax; PP – pneumopericardium; "+" – recovered and discharged home without supplemental oxygen; "-" – deceased

Table 4. Distribution of patients with pneumothorax in regard to its type and resolution

Pneumothorax			
Unilateral	64 (79%)	right-sided	39 (60.9%)
		left-sided	25 (30.1%)
Bilateral	17 (21%)		
Pneumothorax resolution			
Spontaneous	25 (30.9%)		
Thoracic drainage	56 (69.1%)		

Table 5. The underlying etiology in patients with pulmonary air leak syndrome

Underlying disease	n
Perinatal asphyxia	57
TTN	24
MAS	18
Sepsis	15
Pneumonia	10
RDS	9
Complex CHD	4
ICH gr III–IV	4
Multiple congenital anomalies	3
Fetal hydrops	1

TTN – transitional tachypnoea of newborn; MAS – meconium aspiration syndrome; RDS – respiratory distress syndrome; CHD – congenital heart defect; ICH – intracranial hemorrhage

tube, the chest radiography showed pulmonary expansion in 85% of patients, whereas 15% of patients needed thoracic tube revision.

During the ten year period of this study mechanical ventilation (MV) was used to treat 726 neonates. Signs of PALS appeared in 7.2% of all ventilated neonates. Of 87 patients analyzed in the study, radiographic signs of PALS were present in 40.2% of patients who previously did not receive MV. Most commonly used type of MV was synchronized intermittent mandatory ventilation, which was used in 81.5%, while intermittent positive pressure ventilation was used in 18.5% of patients. During the study period, high frequency ventilation was not available at our hospital. The average length of MV was 3.8 ± 7.2 days. In patients with spontaneous pneumothorax, the average length MV was 1.9 ± 2.9 days, whereas in those patients with an underlying pulmonary disease who developed pneumothorax in clinical course the average length of MV was 5.2 ± 9.1 days. There is a statistically significant difference in the length of MV in regard to an underlying condition (U-test 543,5; $p < 0.05$).

The underlying etiology of patients with PALS is presented in Table 5.

Most of our patients (78.2%) had favorable outcome, while 21.8% died. Univariant logistic regression model showed that the variable associated with greater risk for adverse outcome was lower Apgar score at the first minute ($p = 0.001$). The presence of pneumopericardium was at the limit of statistical significance ($p = 0.056$). In group of patients with an unfavorable outcome 63.1% neonates had severe perinatal asphyxia, 26.3% MAS and PPHN, 26.3% had sepsis and/or pneumonia. There were 21% neonates with intracranial hemorrhage (ICH), 21% with complex congenital heart disease (CHD), 15% with congenital anomalies (e.g. Sy Pierre-Robin, polycystic renal dysplasia, tracheoesophageal fistula) and one patient with severe nonimmune fetal hydrops.

DISCUSSION

Pulmonary air leak syndrome appears more commonly in the first month of life than in any other period of life. The overall incidence is estimated to be about 1% of all neonates, although only 10% of patients are symptomatic [1, 3, 6]. It is more common in premature neonates, because of the increased incidence of respiratory distress syndrome (RDS) and need for MV. Pneumothorax is by far the most common type of PALS.

More than two-thirds of all premature labors occur between 34 0/7 and 36 6/7 g. w. [7]. This group of neonates, referred to as "late preterm", stands somewhere between term, mature neonates and those extremely premature, whose prematurity caries well-known risks and long-time complications. This group of neonates experience significantly more morbidity than infants born at term [7, 8]. It is known that these patients have increased incidence of RDS, transitional tachypnoea of newborn (TTN), meconium aspiration syndrome (MAS) with/without persistent pulmonary hypertension of the newborn (PPHN), hypoglycemia, hyperbilirubinemia. When prolonged premature rupture of membranes occurs between 34th and 37th g. w., corticosteroids are not used for fetal lung maturation [7]. An updated Committee Opinion from the American College of Obstetricians and Gynecologists (ACOG), published in August 2017, expands antenatal corticosteroid recommendations to support betamethasone administration to women at high risk for late preterm birth (34th 0/7 – 36th 6/7 weeks) [9]. This is an important point since there is a significant incidence of RDS and TTN in neonates born at this gestational age. It will be of clinical interest to follow the incidence of respiratory problems in this group of neonates in the following years, as we expect it to decrease with the latest update of ACOG recommendations.

In the absence of spontaneous initiation of delivery, there is a lack of multiple hormonal changes, in the fetus and the mother, which induce lung maturation and fetal lung fluid clearance [7, 8, 10]. Thus neonatal adaptation to extrauterine life is more difficult, respiratory physiology is changed and the incidence of RDS and TTN is increased, along with its possible complications, such as PALS, respiratory insufficiency, and pneumonia [8]. It is well documented that delivery by cesarean section is associated with increased risk for pneumothorax, regardless gestational age, especially in the absence of spontaneous initiation of delivery [11]. The fact that there is no significant difference in the mode of delivery in our group of neonates and that there is as much as 54% neonates were delivered by cesarean section is probably due to the fact that all neonates treated in our hospital are transfers (there is no maternity within hospital), so the structure of patients is random.

Higher birth weight carries greater risk of spontaneous pneumothorax in term neonates [12]. This is probably because larger neonates are more commonly born by interventional delivery (vacuum or forceps) and interventions are used prior to complete clearance of lung fluid. During the first few breaths uneven distribution of transpulmonary pressure might precipitate pneumothorax. In our study group, we showed a statically significant difference in birth weight between term and late preterm subgroup of neonates.

It was noticed in the late 70s that primary spontaneous pneumothorax appears more often in term and postterm neonates, especially in the presence of perinatal asphyxia, difficult and prolonged vaginal delivery, resuscitation after birth, presence of blood in airways, meconium in the airways at first aspiration after birth [13]. Therapeutic measures such as bag and mask ventilation in delivery room and resuscitation are well known risk factors for PALS [14]. Most patients with primary spontaneous pneumothorax do not have symptoms or have only mild symptoms and require no treatment. In clinical practice, the majority of these neonates are managed by supplemental oxygen. Most of our patients (62.2%) received oxygenotherapy prior to admission to our hospital. The signs of respiratory distress in the maternity wards resulted in supplying oxygen as the first step of respiratory support in these neonates. This stands along with the fact that 70.1% of them had signs of increased work of breathing and 60.9% were tachypnoeic. A group of Canadian authors showed an interesting result that oxygenotherapy in term neonates with primary spontaneous pneumothorax does not shorten the time to its complete resolution [15].

Respiratory insufficiency occurs in late preterm and term neonates as one of the complications of perinatal asphyxia [8]. In the most severe cases of perinatal asphyxia chronic mechanical ventilation might be needed. In our group of patients, 65.5% were diagnosed with perinatal asphyxia.

Unfavorable outcome was observed in 21.8% of our patients, which can be explained by high percentage of patients with severe perinatal asphyxia and associated conditions that increase the risk of death. In the group of patients with unfavorable outcome, as many as 63% had severe perinatal asphyxia. This result stands in favor of the fact that a lot of effort must be put in preventing perinatal asphyxia in developing countries, as this is an important risk factor for both respiratory insufficiency, complications but also for and an unfavorable outcome.

Several pulmonary diseases such as RDS, TTN, MAS, pulmonary hypoplasia, pneumonia, increase the risk of PALS [16, 17]. Some of these were the most common underlying pulmonary diseases in our group of neonates, as they are frequent respiratory pathology in neonates born at term or late preterm gestation.

MV increases the risk for PALS. The use of surfactant in prevention and treatment of RDS along with modern concept of "gentle" MV significantly decrease the risk of PALS during MV of a neonate [18]. The wide use of nasal continuous positive airway pressure (nCPAP) and nonivasive ventilation diminishes number of neonates who require intubation and conventional MV, thus decreasing the risk of PALS. The use of high frequency ventilation in prevention and treatment of PALS is attracting more attention of neonatologists in the previous years [19]. Conventional MV was the only MV mode available in our hospital for most of the time during the ten-year period of this study, so we could not compare the incidence of PALS in regard to different respiratory support types.

Neonates with asymptomatic pneumothorax without underlying pulmonary disease do not require specific treatment. Needle aspiration with angiocatheter would be an acceptable, less invasive but efficient treatment modality, especially in a patient with mild symptoms, but there is, so far, only one small randomized trial to support its use in neonates [20]. In 69% of our patients, pneumothorax was treated by thoracic underwater drainage. The duration of thoracic drainage was statistically significantly longer in patients with pneumothorax due to an underlying disease, compared to those with spontaneous pneumothorax. Other invasive procedures commonly used in children and adults, such as video-assisted thoracoscopic surgery, are not used in neonates [21].

Statistical analysis distinguished two factors which are connected to an unfavorable outcome: low Apgar score in the first minute and the presence of pneumopericardium. We believe that the former result is of great clinical importance, as pneumopericardium is seen almost exclusively in patients on mechanical ventilation, so its appearance would demand increased vigilance of clinicians. We believe that our results are to be interpreted in the context of our study design which did not include the control group, so the true incidence of PALS in a larger sample of neonates could not be estimated.

CONCLUSION

Pulmonary air leak syndrome in neonates is a life-threatening condition and a medical emergency that requires prompt treatment. Wide use of surfactant in prevention and treatment of RDS, modern concept of noninvasive respiratory support and "gentle" mechanical ventilation lead to an important decrease in the incidence of PALS. A lot of effort should be put into perinatal asphyxia prevention, as it is an important risk factor for PALS in the group of term and late preterm neonates.

Conflict of interest: None declared.

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Плућни синдром цурења ваздуха код терминске и предтерминске новорођенчади

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САЖЕТАК

Увод/Циљ Плућни синдром цурења ваздуха је чешћи у неонаталном узрасту него у било ком периоду живота и његово благовремено препознавање и лечење спада у најхитнија стања у медицини. Приказујемо резултате терцијарног медицинског центра у лечењу плућног синдрома цурења ваздуха код терминске и предтерминске новорођенчади.

Методе Студијом су обухваћена сва новорођенчад рођена између 34. 0/7 и 41. 6/7 гестацијске недеље која су током периода 2005–2015. лечена од плућног синдрома цурења ваздуха на Одељењу неонаталне интензивне неге Института за здравствену заштиту мајке и детета Србије. Анализиране су антропометријске карактеристике, присуство перинаталне асфиксије, примена и тип респираторне потпоре пре пријема, радиографија грудног коша, тип плућног синдрома цурења ваздуха, начин лечења, примарна етиологија болести и коначан исход.

Резултати Анализирано је 87 новорођенчади просечне гестацијске старости 38,1 ± 1,9 гестацијских недеља, просечне порођајне телесне масе 3182,5 ± 55,5 *g*. Њих 47 (54%) рођено је царским резом, док је 40 (46%) рођено природним путем. Пре пријема, оксигенотерапија је примењивана код 62,1% новорођенчади, назални континуирани позитивни ваздушни притисак код 4,6%, а конвенционална механичка вентилација код 21,8% новорођенчади. Начин порођаја као ни врста респиратоне потпоре примењиване пре пријема у неонаталну интензивну негу нису статистички значајно утицали на појаву знакова плућног синдрома цурења ваздуха ($p > 0,05$). Највећи број болесника имао је пнеумоторакс (93,1%), који је најчешће био једнострани (79%). Дужина трајања механичке вентилације статистички је значајно утицала на појаву плућног синдрома цурења ваздуха ($p = 0,015$). Низак индекс бодовања по Апгаровој у првом минуту и присуство пнеумоперикарда су предиктивни фактори за неповољан коначни исход лечења.

Закључак Побољшање стратегије механичке вентилације и смањење учесталости перинаталне асфиксије код терминске и предтерминске новорођенчади могли би да допринесу смањењу учесталости плућног синдрома цурења ваздуха у овој групи болесника.

Кључне речи: пнеумоторакс; новорођенче; респираторна инсуфицијенција; механичка вентилација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Predisposing factors for frostbite – a ten-year retrospective study

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SUMMARY

Introduction/Objective Frostbite is a cold-induced injury of the tissue caused by the freezing of intra- and extracellular water, and characterized by thrombosis and ischemic necrosis. Although individual, socioeconomic, and environmental factors are all considered fundamental determinants of human health in general, their role in the occurrence of frostbite remains largely unknown. The aim of this study was to examine the associations among these factors and frostbite for patients in Belgrade, Serbia.

Methods We investigated a total of 24 patients that were hospitalized and treated for frostbites at the Clinic for Burns, Plastic, and Reconstructive surgery, Clinical Center of Serbia, 2008–2017.

Results The majority (88%) of the patients were male, 58% were long-term alcohol consumers, 46% were long-term smokers, and one patient was drug addict. Of the patients, 14 (58%) had no income and depended on government support, 10 (42%) were employed in physical labor or work on the field, and three (13%) of the patients were homeless. Of the 24 frostbite patients identified, deep frostbite accounted for 18 (75%), of whom 17 (70.8%) had an operative outcome. A majority of patients (42%) sustained frostbite when the temperature was in the range of -5–0°C on the date of occurrence.

Conclusion The results of our study showed that individual, social, and environmental factors were important determinants of frostbite. Our results will contribute to existing evidence for risk factors related to frostbite and will allow for comparisons across countries where these factors have been examined.

Keywords: frostbite; individual factors; socioeconomic factors; environmental factors

INTRODUCTION

Frostbite is a freezing cold thermal injury that occurs when tissues are exposed to temperatures below their freezing point, even above freezing temperature if exposure is prolonged [1]. Pathophysiology of frostbite can be divided into four overlapping pathologic phases: pre-freeze and freeze-thaw, which contribute to direct cellular injury, and vascular stasis and late ischemic phase, which can be described as the carriers of the indirect cellular injuries. The pre-freeze phase consists of vasoconstriction and ischemia. Ice crystal formation and cell disintegration are some of the hallmarks of the freeze-thaw phase. Rewarming leads to reperfusion, causing an inflammatory surge, vascular leak, thrombosis, and embolization that are characteristic of the freeze-thaw phase, but they also largely overlap with the vascular stasis stage. The late ischemic phase results from progressive tissue ischemia and infarction caused by a cascade of events including inflammation mediated by arachidonic acid [2–5].

In the past, frostbite was a leading cause of devastating casualties in wars. More than 10% of all American casualties were due to cold weather-related injuries in World War II and the Korean War. More than 15,000 amputations for frostbite were performed by the German army alone on the Russian front in the winter of 1942 [6]. Recently, concern about frostbite has grown because it has become more widespread [7]. The prevalence of frostbite among the civilian population increased mostly owing to an increase in the number of homeless people, but also because of greater ease of air travel, participation in winter sports, and more ascents to high altitudes [7, 8, 9].

Predisposing factors for frostbite have been described in many large epidemiological studies conducted worldwide [10, 11, 12]. Studies conducted in United States, Norway, and Finland have linked frostbite to homelessness, improper clothing, atherosclerosis, wound infection, diabetes, smoking, and fatigue [13–16]. In Canada it was reported additional predisposing factors related to frostbite, such as alcohol consumption, smoking, psychiatric illness, vehicular failure, drug misuse, and homosexual intercourse [12, 17]. Recent studies have also provided evidence that prevalence is the highest between the ages of 30 and 49 [18, 19].

Although individual, socioeconomic, and environmental factors are considered fundamental determinants of health in general, factors predisposing to frostbite are less well-known than previously thought [18]. Therefore,

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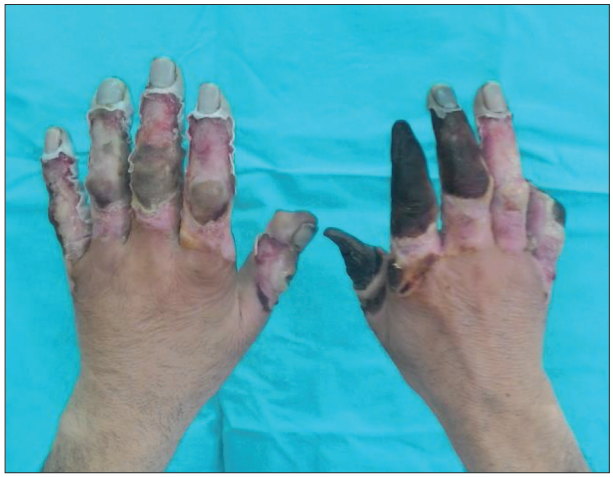

Figure 1. Frostbite of the hands

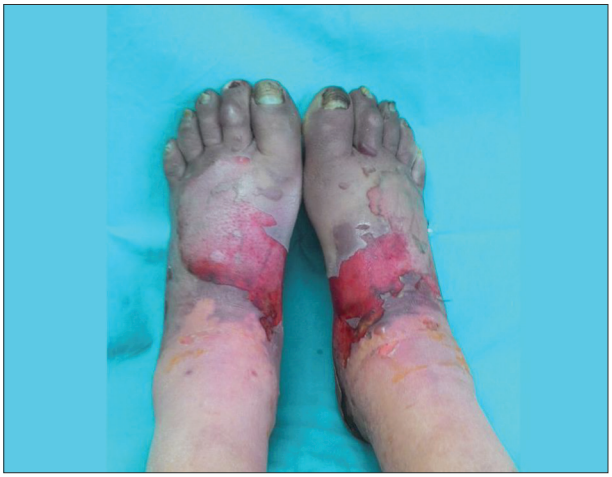

Figure 2. Frostbite of the feet

detailed studies are necessary to determine the predisposing factors influencing frostbite [10, 12, 18]. Therefore, we examined the associations among these factors and frostbite for patients in Belgrade, Serbia. Specifically, we surveyed records of the Clinic for Burns, Plastic, and Reconstructive Surgery, Clinical Center of Serbia for people admitted with frostbite in the period 2008–2017, and determined its association with individual, socioeconomic, and environmental factors. To the best of our knowledge, predisposing factors for frostbite have never been examined in any epidemiological study in Serbia.

METHODS

Study population

The study protocol was approved by the Ethics Committee of the Clinical Centre of Serbia, Belgrade, Serbia. The sample comprised all patients admitted to the Clinic for Burns, Plastic, and Reconstructive Surgery at the Clinical Center of Serbia during the period 2008–2017 with frostbite as primary or secondary diagnosis. A total of 24 patients were treated during the period of this study. The data were collected from the hospital discharge register. Each record contained information on the patient's sex, age, place of residence, employment, relationship status, primary or secondary diagnosis, habits such as smoking, alcoholism and drug abuse, date and place of occurrence of the injury, time between the injury and medical examination, and the length of the hospital stay. One patient was admitted two times for frostbite; only first admissions were included in this research.

Climatic data

To represent the temperatures at which frostbite had been sustained, we used the lowest daily temperature recorded by the closest weather station from the location of the injury on the day of frostbite. Mean daytime temperatures were classified in intervals -20–-15°C; -15–10°C; -10–-5°C; -5–0°C; and 0–5°C.

Statistics

We used descriptive statistics. This method simply described the basic features of the data.

RESULTS

Characteristics of the patients

A majority of the patients (88%) were male with the mean age of 49 years. The mean age of the female patients was 47 years. Frostbite was found to be most common (54%) in patients between 30 and 55 years of age. In 46% of the patients, frostbite was the only diagnosis, whereas 33% were also diagnosed with mental disease, 13% were diagnosed with cardiovascular disease, and 13% had other diagnoses such as diabetes mellitus, dementia, and hepatorenal syndrome. A total of 58% were long-term alcohol consumers, 46% were long-term smokers, and one patient (4,2%) was a drug addict (Table 1). Of the 24 frostbite patients identified, deep frostbite accounted for 18 (75%), of whom 17 (70.8%) had an operative outcome (Figure 1, Figure 2). Amputation occurred in 15 (62.5%) deep injuries and debridement in two (8.4%).

A majority of patients (46%) had no income and depended on government support. The percentage of patients employed was 29%, usually doing physical labor or farming. Seven patients lived alone, five with families, and four patients were homeless. In relation to the affected part of the body, 15 patients had frostbite on the feet, six on the hands, two on both the feet and the hands, and one patient had frostbite on the hands as well as other parts of the body such as ears and knees (Table 1). The average time between the injury and medical examination was 12 days. The average hospital stay was 36 days.

Frostbite and temperature

A majority of patients (42%) sustained frostbite when the temperature was in the range of -5–0°C on the dates of

Table 1. Sex, age, employment, comorbidities, habits, and affected parts of the body

Sex	Number	Percent
Female	3	13%
Male	21	88%
Age		
18–30	2	8%
31–55	13	54%
55+	9	38%
Employment		
Employed	7	29%
Unemployed	11	46%
Retired	3	13%
Unknown	3	13%
Co-morbidities		
Cardiological	3	13%
Mental Illness	8	33%
Neurological illness	3	13%
Habits		
Alcohol	14	58%
Cigarettes	11	46%
Drugs	1	4%
Affected part of the body		
Hand	6	25%
Feet	15	63%
Hands & Feet	2	8%
Hands, ears & knees	1	4%

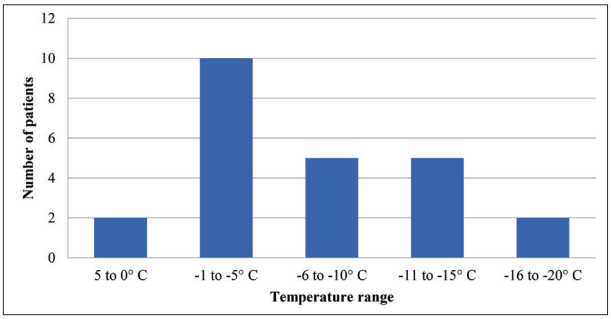

Figure 3. Injury occurrence according to temperature range

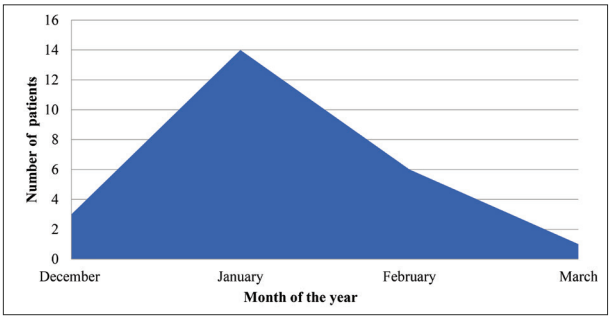

Figure 4. Injury distribution by month

occurrence of the injury (Figure 3). Figure 4 summarizes the climatic pattern and the number of cases per month. The number of patients was highest in January (58%), high in February (25%) and December (12%), and only one case (4%) was seen in March. No patients reported the presence of moisture or immersion of the exposed part of the body to the cold.

DISCUSSION

The results of the study extended work on examining the factors predisposing to frostbite by evaluating its association with individual, socioeconomic, and environmental factors. Our results were similar to those reported by others in some respects. In our study, the majority of patients abused alcohol, and the most severe cases of frostbite were among alcoholics. This is in line with the results obtained by Conway et al. [20], who noted that 27% of all frostbite victims in Alaska were alcoholics. Likewise, Fabian et al. [12] in a 10-year long retrospective study reported that among the top risk factors for frostbite was alcohol abuse.

Our results showed that 33% of the patients suffered from mental illness, generally mirroring previously findings by Kappes et al. [21], who concluded that next to alcohol abuse, mental disorders were the most significant determinants of extensive frostbite. Pinzur and Weaver [22] who found that patients suffering frostbite had significant discrepancies in the percentages of concomitant psychiatric diseases compared with what had been expected. Similarly, recent studies have also shown that large numbers of patients with frostbite have psychiatric illnesses [10].

The study has also revealed that the majority of patients were between 30–55 years old. In contrast, previous research has shown that frostbite is most common among the elderly and young children [23]. Although this grouping seems intuitively correct, recent epidemiological studies have classified adults aged 30–49 years as the group at high risk of frostbite [18, 24]. Moreover, past research has indicated that males are more frequently affected by cold-induced injuries than females, which is also consistent with our results [10, 12, 18, 25, 26]. The incidents reported in these studies are strongly related to these findings, as males constituted a vast majority of subjects.

Furthermore, the results showed that the majority of patients had no income and depended on government support. This finding strongly correlated with many previous studies that have linked frostbite to poverty [22]. For example, according to Pinzur and Weaver [22], the frostbite observed in urban areas in the United States typically occurred among the poor and homeless. Finally, in our study, all patients had frostbite on the feet and hands. These results are consistent with studies that revealed almost unanimous evidence that these anatomical areas were most at risk of sustaining frostbite [10, 27, 28]. The feet and hands are considered sites for 90% of frostbite-related injuries, with the feet the most commonly affected [10].

Our results also showed that frostbite occurrence (42%) was highest at temperatures -5–0°C. However, Juopperi et al. [18] noted in their study in Finland that the incidence of frostbite increased at temperatures below -15°C in Helsinki. It is possible that as the average temperature in Serbia in winter are higher than those in Finland, Serbians may

not be aware of the effects of cold weather, consequently they do not dress adequately, so they may be less qualified at protecting themselves. Inadequate clothing has previously been confirmed as a risk factor for acquiring frostbite in many studies [29].

Certain limitations of our results should be considered. First, the study sample was a consecutive series of patients attending only one clinic in Belgrade during the observed period. The findings obtained in such settings cannot be easily generalized. Second, we collected data from a small number of patients that had been hospitalized, and did not analyze data for patients from outpatient departments who had small or superficial frostbite. But even in Finland, where temperatures are very low in winter Koljonen et al. [30] noted that cases of hospitalization for frostbite were rare. Future work should include both hospitalized patients and those in outpatient departments from all clinics for burns, and plastic and reconstructive surgeries in Serbia. Notwithstanding these limitations, the results of our study showed that many factors determined frostbite in Serbia.

CONCLUSION

This study was added to work that examined individual, social, and environmental factors influencing frostbite. Our data indicated that these factors were important determinants of frostbite. This was noteworthy for several reasons. First, our results contributed to existing evidence for risk factors related to frostbite. Second, the results of our study will allow for comparisons across countries where these factors have been examined. Further, it will help us better understand the issue and allow for greater awareness of frostbite, especially among the public in Serbia and, hopefully, will help prevent future cases. Finally, and more broadly, the factors related to frostbite that were examined provides researchers with additional tools for capturing the evolution of, as well as differences and similarities among, these factors at the global level.

Conflict of interest: None declared.

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Фактори који утичу на настанак смрзотина – десетогодишња ретроспективна студија

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САЖЕТАК

Увод/Циљ Смрзотине су повреде настале смрзавањем интраћелијске и екстраћелијске течности које доводи до тромбозе и исхемијске некрозе. Иако се лични, социоекономски фактори, као и фактори средине сматрају важним детерминантама људског здравља генерално, њихов утицај на смрзотине у великој мери је још увек непознат.
Циљ ове студије био је да се испита утицај ових фактора на настанак смрзотина у Београду (Србија).

Методе У овој студији је учествовало укупно 24 болесника хоспитализована и лечена на Клиници за опекотине, пластичну и реконструктивну хирургију Клиничког центра Србије, у периоду између 2008. и 2017. године.

Резултати Већина болесника (88%) били су мушкарци, 58% болесника били су дугогодишњи алкохоличари, 46% болесника били су дугогодишњи пушачи и један болесник је био зависник од дроге. Четрнаест (58%) болесника није имало приходе и зависило је од подршке државе, 10 (42%) болесника су били физички или пољопривредни радници и три (13%) болесника су била бескућници. Од 24 испитивана болесника дубоке смрзотине је имало 18 (75%) њих, од којих је 17 (70,8%) оперисано. Највећи број болесника (42%) задобио је смрзотине када је температура ваздуха била између -5°C и 0°C.

Закључак Резултати нашег истраживања показали су да индивидуални фактори, социјални фактори, као и фактори средине представљају важне детерминанте код настанка смрзотина. Наши резултати допринеће постојећим доказима о факторима ризика који се односе на смрзотине и омогућиће поређења у земљама у којима су ови фактори испитивани.

Кључне речи: смрзотине; лични фактори; социоекономски фактори; фактори средине

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analysis of the applied technique of intravenous anesthesia for in vitro fertilization in obese and patients with normal body mass index

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SUMMARY
Introduction/Objective In this study, the effects of applied anesthetic techniques were investigated in a retrospective analysis of obese patients and those with normal body mass index undergoing *in vitro* fertilization, using bispectral index as an indicator of anesthetic depth.
Methods In total 116 patients with normal body mass index were allocated to group N. Another 116 patients with body mass index > 30 kg/m^2 were allocated to group O. Anesthetic protocol comprised midazolam for premedication, diclofenac for pre-emptive analgesia, propofol for induction and maintenance, alfentanil for analgesia, suxamethonium for muscle relaxation. We recorded and compared the monitored parameters using t-test and χ^2 test.
Results Procedure duration and recovery time were significantly longer in O group ($p < 0.01$). There is a statistically significant difference ($p = 0.000181$) in the number of patients requiring mechanical ventilation after induction of anesthesia. Propofol consumption was significantly higher ($p < 0.0001$) in O group (2.7 ± 1.6 mg/kg) as compared to group N (2.1 ± 0.4 mg/kg). The incidence of postoperative nausea and vomiting was observed in six patients in N group (5.17%) and nine patients in O group (7.76%). Pain intensity was found higher in group O compared to group N ($p < 0.0001$). Assessment of patients' sedation using verbal scale reported no statistically significant difference between N and O groups ($p = 0.2548$).
Conclusion Induction and maintenance of anesthesia in obese patients results in increased consumption of propofol and the need for muscle relaxation. The statements of the patients who underwent the procedure under intravenous propofol and alfentanil serve as the best recommendation for clinical practice.
Keywords: oocyte retrieval; pain; propofol; alfentanil; body weight

INTRODUCTION

In vitro fertilization (IVF) is an assisted reproductive technology characterized by letting the fertilization of male and female gametes (sperm and egg) occur outside the female body, in the laboratory; created embryos are then transferred into the woman's womb. Stages in IVF procedure are as follows:

- Indications for IVF and preparation for treatment,
- Ovulation induction and monitoring,
- Oocyte retrieval,
- Insemination and fertilization,
- Embryo-transfer.

The role of anesthesiologist is associated to the phase of oocyte retrieval with follicle aspiration. In this stage of the procedure, it is it is necessary to induce analgesia for pain relief, and in this way to provide the optimal conditions for the gynecologist to perform the procedure.

Oocyte retrieval involves direct ultrasound guidance, i.e. a needle is passed through the top of the vagina to reach the follicles. Pain during oocyte retrieval is caused by the puncture of the vaginal skin and ovarian capsule by the aspirating needle, as well as manipulation within the ovary during the entire procedure [1]. The number of follicles and duration of the oocyte retrieval procedure may affect the pain intensity. Single follicle aspiration would take lesser time and cause less pain as compared to multiple follicle aspirations [2]. In addition, the pain intensifies with difficult ovarian access (for instance congenital and acquired anomalies, obesity, etc.) that requires external compression of the lower anterior abdominal walls external abdominal compression. Insufficiently deep anesthesia in these cases can lead not only to the onset of intense pain but also to the reflex movements of patients that can disturb manipulation of aspiration needle and the whole procedure.

Obese patients undergoing IVF present a challenge not only for gynecologists, but also for anesthesiologists, who are to provide adequate anesthesia to make transvaginal oocyte retrieval a safe and effective procedure. Obesity is often accompanied by a series of possible complications on cardiovascular and respiratory systems, increased incidence of

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thrombosis, difficulties related to airway management and the more emphasized adverse pathophysiological effects of the gynecological position [3]. Varieties of anesthetic techniques and modalities have been used in the history of IVF. The procedure necessitates a short-acting anesthetic approach with minimal side-effects. The various anesthetic modalities used for transvaginal oocyte retrieval include monitored anesthesia care, conscious sedation, general anesthesia, regional anesthesia, local injection as a paracervical block, epidural block, subarachnoid block, total intravenous anesthesia, patient-controlled analgesia, and acupuncture [4, 5, 6].

In our study, we investigated the effects of applied anesthetic techniques using propofol and alfentanil (hemodynamic and respiratory stability of patients, the occurrence of perioperative complications associated with anesthetic technique, duration of intervention, anesthetic consumption per patient, length of stay in post-anesthesia care unit, presence and intensity of pain after intervention, postoperative nausea and vomiting, degree of patient satisfaction with anesthesia) in a retrospective analysis of anesthetic and post-anesthetic records of obese and patients with normal body weight undergoing IVF, using bispectral (BIS) index as an indicator of anesthetic depth.

METHODS

The study was conducted in accord with standards of the institutional Committee on Ethics of the Faculty of Medicine Priština – Kosovska Mitrovica and Spebo Medical fertility clinic in Leskovac. Written consents to the administration of intravenous anesthesia were obtained from the patients. The study (retrospective, randomized) included subjects who underwent IVF in the Spebo Medical specialist medical center for fertility treatment in the period 2010–2017. A total of 950 patients with normal BMI (18.5–24.9 kg/m^2) were recorded to have undergone IVF procedure under intravenous anesthesia with propofol and alfentanil. Of these, 116 subjects were randomly assigned following simple randomization procedures (computerized random numbers) to group N (normal BMI). In the same timeframe (2010–2017), 184 patients with BMI > 30 kg/m^2 received intravenous propofol – alfentanil during IVF procedure. Of them, 116 were included in the study and assigned to group O (obese). Data analysis was performed for each patient based on medical records, anesthesia charts, and post-anesthetic monitoring sheets. The anesthesia chart for oocyte retrieval procedure was completed by the anesthetist who administered intravenous anesthesia; whereas the sheets of post-anesthetic monitoring were completed by another anesthetist the patient was handed over to on admission to the post-anesthesia care unit (PACU). All patients were classified according to the American Society of Anesthesiologists (ASA) classification system I–II. Age varied from 18 to 45. The study excluded patients with cardiorespiratory disorders, diabetes, thyroid disorders, chronic opioid and sedative use, allergic reactions to administered anesthetics, opioids, sedatives and nonsteroidal anti-inflammatory drugs.

Anesthetic protocol

All patients underwent a uniform anesthetic protocol. The minimum fasting period was four hours prior to the procedure. Patients preoperatively received low molecular weight heparin for the prevention of thromboembolism**.** A cubital vein cannula was used to administer premedication.

Hydration was provided by continuous infusion of Ringer lactate solution (10 ml/kg body weight [b. w.]). After positioning, the patient is linked to the mandatory standard monitoring for this type of intervention listed below. After recording the monitoring parameters from pre-induction stage, patients were premedicated with 0.02 mg/kg b. w. intravenous midazolam and 1 mg/kg b. w. diclofenac sodium with 100 ml saline infusion. Anesthesia was induced with propofol 2 mg/kg b. w. and alfentanil 0.01 mg/kg b. w. (Table 1).

Additional propofol was administered to maintain BIS values within the target range (40–60). When needed, muscle relaxation was achieved by intravenous administration of suxamethonium chloride.

In the incidence of apnoea after induction of anesthesia, patients were mechanically ventilated through a facemask or a cuffed oropharyngeal airway with tidal volume of 8 ml/kg b. w. The inspiratory mixture of oxygen and medical air delivered the inspired oxygen concentration of 40% (FiO_2 0.4).

Monitoring

The standard monitoring included: BIS index, pulse oximetry (SaO_2), level of (partial pressure) of carbon dioxide released at the end of expiration ($EtCO_2$), peak inspiratory pressure (P_{peak}), plateau airway pressure (P_{plato}), tidal volume (Vt), mean arterial blood pressure (ABP) and electrocardiography (EKG). $EtCO_2$, P_{peak}, P_{plato} and Vt were determined only in patients where intermittent positive-pressure

Table 1. Anesthetic protocol

Premedication and *preemptive analgesia*		Induction of anesthesia		Maintenance of anesthesia	
Drugs	Dose (mg/kg b. w.)	Drugs	Dose (mg/kg b. w.)	Drugs	Dose (mg/kg b. w.)
Midazolam	0.02	Propofol	2	Propofol	0.5
Diclofenac	1	Alfentanil	0.01	Suxamethonium chloride	1.5
Ringer's solution				10 ml/kg b. w.	

b. w. – body weight

Table 2. Demographic characteristics, ASA affiliation, procedure, and recovery time

Variables	Group N	Group O	p-value (t-test)
Age (years ± SD)	34.2 ± 8.7	33.5 ± 8.5	0.4471
Body weight (kg ± SD)	53.4 ± 14.7	73.5 ± 23.4	< 0.0001
Body height (cm ± SD)	162.7 ± 17.8	163.9 ± 13.8	0.5055
ASA I affiliation	80 (68.96%)	41 (35.34%)	0.003828 (χ^2 test)
ASA II affiliation	36 (31.04%)	75 (64.66%)	0.002182 (χ^2 test)
Procedure time (min. ± SD)	17.6 ± 7.3	24.2 ± 5.6	< 0.0001
Recovery time (min. ± SD)	8.5 ± 4.2	15.3 ± 3.1	< 0.0001

ASA – American Society of Anesthesiologists; SD – standard deviation; min. – minutes
p > 0.05 – non-significant
p < 0.05 – significant
p < 0.01 – highly significant

ventilation (IPPV) was applied. Parameters were analyzed at following intervals: T_0 – baseline, T_1 – after induction to anesthesia, and T_2 – at the end of the procedure. Clinical parameters were measured by vital sign monitor (BIS™ Complete 2 Channel Monitor, Covidien, Minneapolis, MN, USA) (Medtronic and Monitor Infinity Gamma XL, Dräger, Lübeck, Germany) and anesthesia machine (Fabius Tiro Anesthesia Machine, Dräger).

BIS index is processed electroencephalographs monitor which measures the effects of sedatives and anesthetics on the brain; a new vital sign that allows clinicians to deliver anesthesia with more precision and to assess and respond more appropriately to patients changing condition during surgery [7]. The BIS monitor provides a single number, which ranges from 0 to 100 where the value between 40 and 60 indicates an appropriate level for general anesthesia [8].

Recovery Room / Post-Anesthesia Care Unit

Post-anesthetic monitoring included the following parameters:

- The need for additional analgesia;
- Presence and intensity of pain (we used a modified visual analogue scale (VAS) where pain descriptors were assigned an intensity value. Categories proposed were: 0 – no pain, 1–30 mild, 40–60 moderate, 70–90 severe, 100 – extreme);
- Presence of postoperative nausea and vomiting (PONV);
- The need for administration of ondasetron;
- The length of stay in PACU;
- The overall patient satisfaction with analgesia and sedation (overall anesthetic experience) was assessed by a second anesthesiologist before discharge using a 4 – point verbal scale ranging from very satisfied to very dissatisfied (1 – very dissatisfied, 2 – dissatisfied, 3 – satisfied, 4 – very satisfied).

Statistical Analysis

The analysis of obtained data was performed using the IBM SPSS Statistics software for Windows (IBM Corp. Version 22.0. Armonk, NY, USA) as well as Microsoft Excel 2010. Descriptive statistics was used to determine the relative numbers and measures of the central tendency: the arithmetic mean (X), a measure of variability, standard deviation (SD) and the relative proportions (percentages).

The monitored parameters were recorded and compared using the Student's t-test and χ^2 test. P-values > 0.05 were considered statistically non-significant, p-values < 0.05 were considered statistically significant, and p-values < 0.01 were considered statistically highly significant for all comparisons.

RESULTS

Data analysis reported no statistical difference (p > 0.05, t-test; Table 2) between the groups with respect to age (group N: 34.2 ± 8.7; group O: 33.5 ± 8.5) and height (group N: 162.7 ± 17.8 cm; group O: 163.9 ± 13.8). The χ^2 test revealed a significant difference (p < 0.01; Table 2) between the two groups in the ASA classification. There was a statistically significant difference (p < 0.01, t-test; Table 2) between the groups with respect to weight, length of surgery, and recovery time.

Table 3 shows the values of the BIS index, hemodynamic and respiratory parameters (ventilation and oxygenation) obtained during monitoring intervals (T). A comparative analysis (t-test) between the tested groups reported a statistically significant difference, except for the BIS index and pulse values at the T_0 time interval (p > 0.05). The χ^2 test reported a statistically significant difference (p = 0.000181) with respect to the number of patients requiring IPPV for anesthesia maintenance after introduction. Mechanical ventilation was delivered in 82 patients of group N, compared to 33 patients of group O.

Propofol consumption was statistically higher (p < 0.0001, t-test; Table 4) in group O (2.7 ± 1.6 mg/kg b. w.) compared to group N (2.1 ± 0.4 mg/kg b. w.). Twenty-four patients in group O required muscle relaxation with suxamethonium to create the state of complete immobilization and optimal conditions for the performance of transvaginal aspiration of ovarian follicles by a gynecologist. In contrast, in group N, suxamethonium was administered to only five patients (p = 0.000852, χ^2 test; Table 4).

After induction to anesthesia with propofol (2 mg/kg b. w., intravenous), sufficient spontaneous breathing was preserved in 18 patients in group N and 46 in group O (p = 0.001855, χ^2 test; Table 5). Assisted ventilation was

Table 3. Bispectral index, hemodynamic and parameters of ventilation and oxygenation through determining time intervals (T)

T – intervals and parameters	T_0			T_1			T_2		
	Group N	Group O	p-value (t-test)	Group N	Group O	p-value (t-test)	Group N	Group O	p-value (t-test)
BIS index	98.4 ± 1.7	97.7 ± 3.8	0.0714	45.3 ± 5.9	54.1 ± 7.4	< 0.0001	48.4 ± 6.7	57.8 ± 8.9	< 0.0001
Pulse	96.4 ± 12.3	93.1 ± 15.7	0.0761	65.4 ± 11.4	73.5 ± 13.1	< 0.0001	73.8 ± 15.7	83.9 ± 16.3	< 0.0001
ABP_{mean} (mmHg)	82.7 ± 14.1	93.4 ± 11.5	< 0.0001	67.2 ± 9.4	84.4 ± 11.3	< 0.0001	73.9 ± 14.6	91.5 ± 15.2	< 0.0001
SaO_2 (%)	99.4 ± 0.7	95.3 ± 1.7	< 0.0001	98.5 ± 1.2	95.6 ± 2.1	< 0.0001	98.7 ± 1.7	95.4 ± 2.4	< 0.0001
$EtCO_2$ (mmHg – IPPV)	-	-	-	28.4 ± 6.3	35.1 ± 5.5	< 0.0001	27.9 ± 4.8	36.6 ± 4.9	< 0.0001
P_{peak} (mbar – IPPV)	-	-	-	11.3 ± 2.4 (n – 82)*	17.6 ± 1.9 (n – 33)*	< 0.0001	12.5 ± 1.6 (n – 82)*	18.6 ± 2.3 (n – 33)*	< 0.0001
P_{plato} (mbar – IPPV)	-	-	-	9.8 ± 1.8 (n – 82)*	14.4 ± 1.6 (n – 33)*	< 0.0001	10.4 ± 1.6 (n – 82)*	16.5 ± 1.9 (n – 33)*	< 0.0001
Abdominal pressure							11 (9.48%)	46 (39.6%)	0.000029 (χ^2 test)

T_0 – baseline; T_1 – after induction to anesthesia; T_2 – at the end of the procedure; Group N – patients with normal body mass index; Group O – obese patients; BIS index – bispectral index; ABP_{mean} – arterial blood pressure; SaO_2 – pulse oximetry; $EtCO_2$ – carbon dioxide released at the end of expiration; IPPV – intermittent positive pressure ventilation; P_{peak} – peak inspiratory pressure; P_{plato} – plateau airway pressure;
p > 0.05 – non-significant;
p < 0.05 – significant;
p < 0.01 – highly significant

Table 4. Total anesthetics and drugs consumption

Variables (mg/kg b. w.)	Group n	Group O	p-value (t-test)
Propofol	2.1 ± 0.4	2.7 ± 1.6	< 0.0001
Alfentanil	0.01	0.01	-
Suxamethonium chloride	1.5 (n – 5)	1.5 (n – 24)	For n: 0.000852 (χ^2 test)
Midazolam	0.02	0.02	-
Diclofenac	1	1	-
Solution of lactated Ringer	10 ml/kg b. w.	10 ml/kg b. w.	-

Data are presented as mean ± standard deviation or n (number of patients);
p > 0.05 – non-significant;
p < 0.05 – significant;
p < 0.01 – highly significant

required in 16 patients in group N and 37 patients in group O (p = 0.009063, χ^2 test; Table 5). The depressive effect of propofol on the respiratory center caused apnoea in 82 patients of group N and 33 in group O (p = 0.000161, χ^2 test; Table 5) and here it was necessary to perform IPPV using an anesthesia machine ventilator.

Anesthesia and controlled ventilation were delivered via a facemask. After induction to anesthesia, hypopharyngeal obstruction from tongue displacement was handled with the use of oropharyngeal airway in 24 patients in group N and 88 in group O (p < 0.01, χ^2 test; Table 5). At the end of the surgery, no statistical differences were reported with respect to applied mode of ventilation. There was no need for endotracheal intubation or placement of a laryngeal mask to maintain an open airway.

Post-operatively, additional analgesic administration (one intravenous dose) was required in 13 (11.2%) patients in group N. In group O, an additional intravenous dose of analgesics was required in 48 (39.66%) patients (p = 0.000113, χ^2 test; Table 6).

PONV occurred in six patients (5.17%) in group N and nine (7.76%) in group O after applying ondansetron hydrochloride. Comparison of the obtained data using χ^2 test did not show a statistically significant difference (p = 0.452795; Table 6).

Duration of PACU stay was longer in group O (13.7 ± 6.3 min.) compared to group N (19.6 ± 7.3 min.). Here, the Student's t-test reported a statistically significant difference (p < 0.0001; Table 6).

Measurement of pain intensity after admission and before discharge to PACU, using the combination of visual and numeric analogue scales, reported higher values in group O compared to group N (p < 0.0001, t-test; Table 6).

Scores based on Satisfaction with Anesthesia Scale revealed no statistical significance between the groups (p = 0.2548, t-test; Table 6).

DISCUSSION

The ideal anesthetic technique for IVF should provide good surgical anesthesia with minimal side effects, a short recovery time, high rate of successful pregnancy, and shortest required duration of exposure. The preferred method of anesthesia and analgesia should be individualized [9].

Using BIS monitor to guide anesthetic administration would allow optimization of drug delivery to the individual needs of each patient in order to avoid unnecessarily deep or too light anesthesia due to overdosage or underdosage of the hypnotic medications [10]. BIS values in both groups signifies that increasing depth of anesthesia was associated with a decrease in BIS values and the decreasing level of anesthesia was associated with increasing BIS values [11].

Benzodiazepines are used for premedication, procedural sedation, and supplementation of general or regional anesthesia. A common sequel to intravenous administration of benzodiazepines is anxiolysis and anterograde amnesia. These two main characteristics of these drugs make them suitable for patients undergoing unpleasant or repeated procedures, like oocytes retrieval. In both tested groups, premedication with midazolam was found to be

Table 5. The ventilation model and the way of establishing and maintaining the airway

T – intervals and ventilation	T_0			T_1			T_2		
	Group N	Group O	p-value (χ^2 test)	Group N	Group O	p-value (χ^2 test)	Group N	Group O	p-value (χ^2 test)
Spontaneous breathing	116 (100%)	116 (100%)	1	18 (15.5%)	46 (39.6%)	0.001855	83 (71.6%)	68 (58.6%)	0.341715
Assisted ventilation	-	-	-	16 (13.8%)	37 (31.9%)	0.009063	25 (21.6%)	32 (27.6%)	0.405993
Controlled ventilation (IPPV)	-	-	-	82 (70.7%)	33 (28.4%)	0.000161	8 (6.9%)	16 (13.8%)	0.119869
Face mask	-	-	-	116 (100%)	116 (100%)	1	116 (100%)	116 (100%)	1
Oropharyngeal airway	-	-	-	24 (20.7%)	98 (84.5%)	< 0.01	24 (20.7%)	98 (84.5%)	< 0.01
Laryngeal mask	-	-	-	-	-	-	-	-	-
Endotracheal tube	-	-	-	-	-	-	-	-	-

T_0 – baseline; T_1 – after induction to anesthesia; T_2 – at the end of the procedure; Group N – patients with normal body mass index; Group O – obese patients; IPPV – intermittent positive pressure ventilation;
$p > 0.05$ – non-significant;
$p < 0.05$ – significant;
$p < 0.01$ – highly significant

Table 6. Postoperative outcome measures

Variables	Group N	Group O	p-value
Oocytes retrieved (n ± SD)	10.3 ± 3.1	7.6 ± 2.8	< 0.0001 (t-test)
The need for additional analgesia	13 (11.2%)	46 (39.66%)	0.000113 (χ^2 test)
Postoperative nausea and vomiting	6 (5.17%)	9 (7.76%)	0.452795 (χ^2 test)
Average postoperative VAS pain scores (0–100 mm ± SD) (entrance/exit PACU)	14.7 ± 7.1 / 21.4 ± 12.3	26.8 ± 12.2 / 47.2 ± 13.4	< 0.0001/< 0.0001 (t-test)
Ondansetron hydrochloride	6 (5.17%)	9 (7.76%)	0.452795 (χ^2 test)
Length of PACU stay (min. ± SD)	13.7 ± 6.3	19.6 ± 7.3	< 0.0001 (t-test)
Patient satisfaction score (1–4 ± SD)	3.4 ± 0.5	3.3 ± 0.8	0.2548 (t-test)

SD – standard deviation; min. – minutes; PACU – post-anesthesia care unit;
$p > 0.05$ – non-significant;
$p < 0.05$ – significant;
$p < 0.01$ – highly significant

an adequate means to address fear and anxiety and create optimal conditions for puncture and aspiration of ovarian follicles. Although minimal amounts of this benzodiazepine were found in follicular fluid, no detrimental effects have been proven so far [12]. Furthermore, midazolam enhances the postoperative analgesic effects of diclofenac when used before the onset of noxious stimuli [13].

The pain during oocyte retrieval is caused by the puncture of the vaginal skin and ovarian capsule by the aspirating needle as well as manipulation within the ovary during the entire procedure [14]. Here it becomes customary for the anesthetist to provide adequate pain relief to immobilize the patient and eliminate the danger of piercing any vessels during the process of oocyte retrieval. The ideal pain relief during oocyte retrieval should be effective and safe, easy to administer and monitor, short acting and readily reversible with few side effects [15, 16].

There are animal studies that bring impressive evidence of the efficacy of prior administration of non-steroidal anti-inflammatory analgesics in treatment of inflammatory diseases [17]. Preemptive administration of non-steroidal anti-inflammatory drugs reduces the average perioperative consumption of opioid analgesics. In their retrospective study, Mialon et al. [18] compared two analgesic protocols: paracetamol/alprazolam and nefopam/ketoprofen on IVF outcomes. They found that both groups had similar IVF outcomes and nefopam/ketoprofen protocol enhanced patient comfort without jeopardizing the IVF success rates. Women can be offered adequate pain relief. Opioids are used in oocyte retrieval procedure primarily for their analgesic effects. The most frequently used are fentanyl, alfentanil, and remifentanil, because of their pharmacokinetic profile that enhances fast track anesthesia.

Pethidine is used in some cases as an agent of premedication. The amount of alfentanil is not associated with adverse effects on fertilization rate, embryo development, or clinical pregnancy rate [19]. Both of the groups received propofol for induction and maintenance of anesthesia. Propofol is the most commonly used intravenous anesthetic agent in sedation and general anesthesia. Its pharmacokinetic profile makes propofol anesthetists' first choice. It provides rapid induction and easy maintenance in continuous infusion or fractionated doses.

Several studies investigate the effect of this agent on IVF success with conflicting results [20–25]. Of the studies investigating toxicity, two of them relate propofol with negative effects on the reproductive outcome, and five studies conclude with the opposite result [20–25].

According to these findings, propofol is probably a safe choice, but caution is recommended since Propofol also accumulates in the follicular fluid [24]. Its hemodynamic effect results in a decrease in arterial blood pressure and

pulse [26]. However, in both groups of subjects, this decrease was within physiological limits. Increased values of arterial blood pressure and pulse in obese patients should be associated to increased sensitivity to pain during aspiration of ovarian follicles. This is conditioned by the difficulty in accessing ovaries in obese women, when surgeons often require assistance by compressing the lower abdomen. These additional manipulations can lead to unconscious movement of patients and in this way increase the risk of aspiration needle damaging the surrounding anatomical structures. In order to prevent this, it is often necessary to administer additional dose of propofol and sometimes use short-acting muscle relaxants such as suxamethonium. This may explain the higher consumption of propofol (mg/kg b. w.) and the more frequent use of relaxants in obese patients. The administered induction dose of propofol (2 mg/kg b. w.) in certain patients of both groups, resulted in the cessation of breathing or decreased pulmonary ventilation, to the extent that it was necessary to apply assisted or controlled ventilation.

Propofol is widely used for anesthesia and sedation purposes because of its amnesic effect, fast recovery, and low incidence of nausea and vomiting. Propofol, however, has the shortcoming of severe respiratory depression, including a decrease in ventilatory response to hypoxia and in tidal and minute volumes [27].

The problem of securing and maintaining an open airway has been known. In this study, for the purpose of securing the airway and providing adequate ventilation, it was necessary to use an oropharyngeal tube in almost two-thirds (84.5%) of obese patients. There was no need for laryngeal mask and endotracheal intubation in neither of groups of patients. Delivering controlled ventilation using an anesthesia machine ventilator through the full-face mask with or without the assistance of an oropharyngeal airway was accompanied with statistically higher values of ventilation parameters ($EtCO_2$, P_{peak}, and P_{plato}) in group O compared to group N. Abdominal compression caused an increase in intra-abdominal pressure, cranial displacement of the diaphragm, decrease in lung and chest wall compliance, and an increase in airway resistance, which, paired with obesity, resulted in significantly higher P_{peak} and P_{plato} values in O group.

The difficulty in accessing ovarian follicles in obese women requires additional surgical manipulations, resulting in additional administration of analgesics during the patient's stay at PACU. This may partly explain the higher PONV rate and the need for introducing antiemetics in O group.

As an intravenous anesthetic, propofol shows a rapid rate of metabolism, resulting in quick recovery from anesthesia with few side effects. Because of the low incidence of nausea and vomiting, propofol is commonly used for anesthesia induction and maintenance in ambulatory surgery.

An anesthetic protocol that involved the use of sedatives (midazolam), intravenous anesthetics (propofol) and opioids (alfentanil) resulted in a high degree of patient satisfaction with anesthesia. Developments in medical technology have resulted in a rapid increase in the use of ambulatory surgery. The use of fast- and short-acting anesthetics, analgesics, and muscle relaxants, as well as improved brain monitoring techniques, has reduced anesthetic complications during recovery. Additionally, improvements in surgical techniques have allowed surgeons to perform more invasive surgical procedures and complex medical procedures on an ambulatory basis [28].

CONCLUSION

Intravenous anesthesia with propofol and alfentanil has created adequate conditions for the aspiration of ovarian follicles. Midazolam was found to be the ideal means for premedication and creation of favorable conditions for the patient to undergo the procedure. Preemptive administration of diclofenac reduced the preoperative consumption of alfentanil. During their stay in PACU, these patients experienced mild, or no pain. Induction and maintenance of anesthesia for IVF in obese patients results in increased consumption of propofol and a more frequent need for muscular relaxation. However, the recovery was fast and followed by a low PONV rate. Therefore, the very first assessment of the patients who underwent the procedure under intravenous anesthesia with propofol and alfentanil is the best recommendation for clinical practice.

Conflict of interest: None declared.

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Анализа примењене технике интравенске анестезије за вантелесну оплодњу код гојазних болесница и болесница са нормалним индексом телесне масе

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САЖЕТАК

Увод/Циљ Ретроспективно се анализирала примењена интравенска анестезија пропофолом и алфентанилом у вантелесној оплодњи код гојазних болесница и болесница са нормалним индексом телесне масе коришћењем биспектралног индекса као индикатора дубине анестезије.

Метод Групу *N* сачињавало је 116 болесница са нормалним индексом телесне масе, а групу *O* 116 болесница са индексом телесне масе > 30 kg/m^2. Протокол анестезије састојао се од мидазолама за премедикацију, диклофенака за преемптивну аналгезију, индукције и одржавања анестезије пропофолом, аналгезије алфентанилом, суксаметонијума за мишићну релаксацију уколико је неопходна. Мониторовани параметри били су забележени и упоређивани коришћењем *t*-теста и χ^2 теста.

Резултат Трајање процедуре и опоравак дужи су у групи *O* ($p < 0,01$). Статистички високо значајна разлика ($p = 0,000181$) постоји при упоређивању броја болесница из испитиваних група којима је била неопходна механичка вентилација након индукције у анестезију и њеном одржавању. Потрошња пропофола је статистички значајно већа ($p < 0,0001$) у групи *O* (2,7 ± 1,6 *mg/kg*) у поређењу са групом *N* (2,1 ± 0,4 *mg/kg*). Постоперативна мучнина и повраћање јавили су се код шест (5,17%) болесница групе *N* и девет (7,76%) болесница групе *O*. Интензитет бола је већи у групи *O* у односу на групу *N* ($p < 0,0001$). Вербална скала задовољства болесница анестезијом и седацијом није дала статистичку значајност између група *N* и *O* ($p = 0,2548$).

Закључак Индукција и одржавање анестезије код гојазних болесница резултира већом потрошњом пропофола и потребом за мишићном релаксацијом. Сама оцена болесница примењене технике интравенске анестезије пропофолом и алфентанилом њена је најбоља препорука за клиничку праксу.

Кључне речи: аспирација јајних ћелија; бол; пропофол; алфентанил; телесна тежина

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Hospitalization characteristics of patients with glaucoma in Central and West Serbia

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SUMMARY

Introduction/Objective Glaucoma is a chronic disease that impairs the optic nerve irreversibly and can lead to serious loss of vision and blindness. As the most frequent out of all, primary open-angle glaucoma has a worldwide incidence of 2.4 million.

The objective of this article is to examine the characteristics of glaucoma hospitalization patterns in Central and West Serbia in the 2006–2017 period.

Methods This study was a retrospective analysis of glaucoma hospitalizations in the Kragujevac Clinical Center from 2006 to 2017 (n = 1,751). All hospitalizations were divided according to discharge diagnoses into the following three subgroups: primary open-angle glaucoma, the primary closure glaucoma, and secondary glaucoma and other glaucoma types.

Results The average hospitalization rate for glaucoma is 5/10,000 inhabitants. The lowest rate was recorded in 2013 (1.8/10,000) and the highest in 2015 (9.3/10,000). The rehospitalization rate ranged from 0.5/10,000 in 2013 to 6.9/10,000 in 2015, with an average of 2.4 patients per 10,000. The most common glaucoma was secondary glaucoma and other glaucoma types (44.6%), followed by primary open-angle glaucoma (37.9%) and primary closure glaucoma (17.5%). The average hospitalization length was 6.5 ± 4.9 days and it decreased from the average 9.7 ± 6.5 (2006) to 5.5 ± 3.7 days (2013) ($p < 0.01$) in all glaucoma types.

Conclusion There was a significant reduction of the hospitalization length in all glaucoma types in Central and West Serbia. The hospitalization rates varied with a significant increase since 2013, which is the consequence of the increase in rehospitalization rates.

Keywords: primary open-angle glaucoma; primary closed-angle glaucoma; secondary glaucoma; hospitalization

INTRODUCTION

Glaucoma is a chronic disease that impairs the optic nerve irreversibly and can lead to serious loss of vision and blindness. After cataract, it is the second leading cause of blindness worldwide and is one of the leading causes of preventable blindness [1, 2]. It is estimated that by 2020, about 79.6 million people in the world will have glaucoma and more than 11 million will be consequently bilaterally blind [3]. The annual incidence of primary open-angle glaucoma (POAG) worldwide is 2.4 million. The prevalence of blindness in all types of the disease has been estimated at 5.2 million with three million cases with POAG. POAG is thus a complex and significant public health problem [4].

The two most common clinical forms of glaucoma are POAG and primary angle-closure glaucoma (PACG) [5]. Glaucoma is an asymptomatic disease in many patients. Patients do not know that they have glaucoma because progressive visual field loss is peripheral and typically asymmetric. This allows for overlapping and the compensation from the less damaged visual field of the other eye. Visual field defects can be detected at parametric tests only after 30% of retinal ganglion cells have been lost. The risk factors for POAG include old age, black race, glaucoma family history, diabetes mellitus, arterial blood pressure variations, myopia, and hypermetropia [6–9]. Glaucoma diagnostics requires a detailed clinical examination of the optic nerve and a functional analysis/evaluation of the patient's field of vision. Early treatment of glaucoma patients reduces the risk of progressive damage of a vision field. The prognosis depends on an early diagnosis and adequate treatment, as well as the patient's understanding of his own condition and what comes with the disease [10]. The Preventive Services Task Force in the United States concluded that there was no insufficient evidence on the potential benefits of glaucoma screening at the level of primary health care (prevention of blindness) [11].

The objective of this article is to assess the glaucoma hospitalization reports in the Central and West Serbia regions during the period from 2006 to 2017.

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METHODS

The study was based on hospitalization reports from the Clinical Center of Kragujevac providing data on medical treatment for patients coming from Central and West Serbia, in accord with standards of the institutional committee on ethics. From 2006 to 2017, 1,751 persons were hospitalized for glaucoma treatment. The glaucoma was defined according to the 10th Revision of the International Classification of Diseases: Eyelid Eye Disorders, Lacrimal System and Orbit Disorders (H00–H06). All hospitalized patients were divided according to the major cause of the disease into three subgroups. The first subgroup includes the hospitalized patients discharged with the diagnosis of POAG (H40.1), while the second subgroup was discharged with PACG (H40.2). The third subgroup, named "glaucoma secundaria and other glaucoma (OG)," included patients with congenital glaucoma, glaucoma caused by drugs, injuries and/or other illnesses – H40.3, 4, 5, 6, 8, 9, and H42. Having analyzed the disease trends, the authors decided to focus their research on three intervals within the research period: 2006–2010, 2011–2013, and 2014–2017.

The data from hospitalization reports were entered into a Microsoft Access (Microsoft Corporation, Redmond, WA, USA) database. The data included basic demographic characteristics of the patients, the number and length of their hospitalizations, additional comorbidities, and rehospitalization periods.

The data were analyzed applying the methods of descriptive and analytic statistics. The techniques of descriptive statistics included mean values, variability measures, and structure indicators (percentage). The χ^2 test, i.e. Student's t-test, was used for assessing the significance of categorical data frequency, while one-way ANOVA test was applied for continual data. The statistical significance was selected at $p \leq 0.05$. The data was processed in SPSS, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Basic sample characteristics

During the 2006–2017 period, the Clinical Center of Kragujevac recorded 634,206 hospitalized patients, out of which 14,217 (2.2%) were admitted at the Department of ophthalmology. Glaucoma, as the discharge diagnosis, was present in 2.2% (n = 1,751) of ophthalmology hospitalized patients. The average frequency of glaucoma patients was 5/10,000 inhabitants for the given time period. The lowest rate was in 2013 (1.8/10,000 inhabitants) and the highest in 2015 (9.3/10,000 inhabitants). The rehospitalization rate of glaucoma patients moved from 0.5/10,000 inhabitants in 2013 to 6.9/10,000 in 2015, with the average value being 2.4/10,000 inhabitants. The average age of hospitalized patients was 68.6 ± 12.4 years. Both sexes were almost equally affected (50.5% male and 49.5% female patients). The difference between the sexes was not statistically significant ($t = -0.18$, $df = 941$, $p > 0.05$). The average hospitalization length was 6.5 ± 4.9 days (the range was 1–35 days) and it actually decreased from the average of 9.7 ± 6.5 days recorded in 2006 to 5.5 ± 3.7 days in 2013 ($F = 18.41$, $df = 11$, $p < 0.01$) in all glaucoma types. Comorbidities were present in 120 cases. One additional comorbidity was detected in 51 cases, two in 69 cases, and three or more comorbidities in 20 cases. The comorbidities most frequently originated from vascular system disorders. The most prevalent were OG glaucoma (44.6%), followed by POAG (37.9%), and PACG (17.5%). The analysis of the number of hospitalized patients in all three glaucoma types over the selected time period has revealed that there are some differences between them, but not of statistical significance ($p > 0.05$, Figure 1).

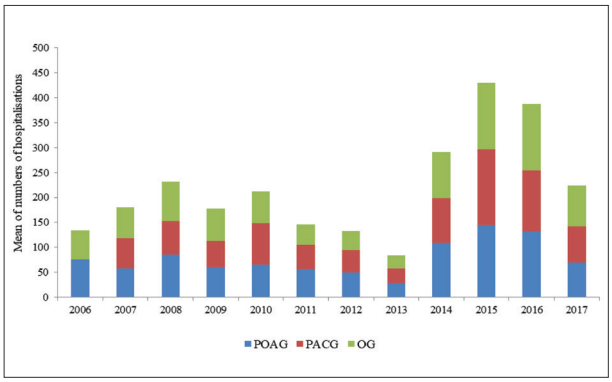

Figure 1. Hospitalization by type of glaucoma, Central and West Serbia, 2006–2017

Basic hospitalization characteristics with respect to glaucoma type

During the 2006–2010 time period, one half of the patients were diagnosed with POAG and they were on average one or two years older than those hospitalized for OG and PACG. Every third male and every fifth female patient were diagnosed with POAG. The hospitalization length was the shortest for those suffering from PACG. One third of all hospitalization periods were actually rehospitalizations. Comorbidities were present in 2% of hospitalizations. During the 2011–2013 period, an overall decreasing trend in glaucoma hospitalizations was recorded. The most prevalent discharge diagnosis was OG, present in 41.3% of cases. The male patients were most commonly hospitalized because of POAG, while the female patients as a result of PACG and OG. In comparison to the previous time period, the number of hospital days increased only in PACG patients. The highest number of rehospitalizations was recorder among OG patients.

From 2014 to 2017, the number of hospitalizations increased due to an increase of rehospitalizations (Figure 2).

OG was the leading cause of hospitalizations and it was a discharge diagnosis in almost every fourth patient of both sexes. The average length of hospitalization decreased from 6.4 to 5.1 days. Comorbidities were present in 12.3% of all hospitalizations (Table 1).

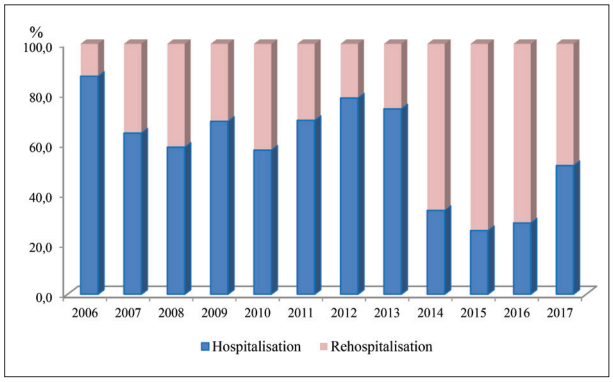

Figure 2. Hospitalization and rehospitalization, Central and West Serbia, 2006–2017

Tabele 1. Hospitalization characteristics of patients with glaucoma, Central and West Serbia, 2006–2017

Variables	2006–2010				2011–2013				2014–2017			
	POAG	PACG	OG	p	POAG	PACG	OG	p	POAG	PACG	OG	p
n (%)	341 (50.1)	57 (8.4)	282 (41.5)	< 0.01	81 (34.2)	58 (24.5)	98 (41.3)	< 0.01	241 (28.9)	191 (22.9)	402 (48.2)	< 0.01
Age (mean ± sd)	69.2 ± 10.4	66.9 ± 16.4	68.4 ± 12.1	> 0.05	70.7 ± 11.1	67.7 ± 14.2	66.4 ± 12.8	> 0.05	71.2 ± 9.8	70.4 ± 9.9	70.5 ± 11.5	> 0.05
Male	212 (31.2)	16 (2.4)	126 (18.5)	< 0.01	49 (20.7)	17 (7.2)	56 (23.6)	< 0.01	118 (14.1)	67 (8)	209 (25.1)	< 0.01
Female	129 (19)	41 (6)	156 (22.9)		32 (13.5)	41 (17.3)	42 (17.7)		123 (14.7)	124 (14.9)	193 (23.1)	
Hospital days (mean ± sd)	8.2 ± 6.1	6.9 ± 3.6	8.5 ± 5.8	> 0.05	5.9 ± 4.5	7.2 ± 6.1	6.3 ± 4.5	> 0.05	4.7 ± 3.3	5.5 ± 3.8	5.2 ± 3.7	< 0.05
Rehospitalizations (n, %)	99 (14.6)	17 (2.5)	105 (15.4)	< 0.01	15 (6.3)	16 (6.8)	31 (13.1)	> 0.05	182 (21.8)	141 (16.9)	240 (28.8)	< 0.01
Comorbidities (n, %)	7 (1)	3 (0.4)	4 (0.6)	> 0.05	1 (0.4)	/	/	> 0.05	36 (4.3)	20 (2.4)	47 (5.6)	< 0.01

POAG – open-angle primary glaucoma; PACG – primary angle-closure glaucoma; OG – glaucoma secundaria and other glaucoma

Basic hospitalization characteristics with respect to glaucoma type: 2006–2010 *vs*. 2011–2013

The number of POAG hospitalizations decreased significantly in the course of 2010 ($\chi^2 = 160.24$, df = 1, $p < 0.01$). This correlates with the decreasing number of rehospitalizations ($\chi^2 = 3.78$, df = 1, $p = 0.05$). The hospitalized patients were in 60% of cases males of similar age. During the given time frames, there was a statistically significant decrease in the average number of hospital days from 8.2 to 5.9 ($t = 3.87$, df = 156.6, $p < 0.01$). In most cases the comorbidities did not occur. The changes that occurred with hospitalized PACG patients were not statistically significant. The number of OG hospitalizations decreased significantly ($\chi^2 = 89.17$, df = 1, $p = 0.01$). Until 2010, there were more male patients, but from 2011 on, female patients started to prevail ($\chi^2 = 4.53$, df = 1, $p < 0.05$). The duration of stays in the hospital was reduced by two days on average ($t = 3.88$, df = 218.9, $p < 0.01$). The rehospitalization rates for OG were similar during both time intervals.

Basic hospitalization characteristics with respect to glaucoma type: 2011–2013 *vs*. 2014–2017

During this period, the number of POAG hospitalizations increased significantly ($\chi^2 = 79.51$, df = 1, $p < 0.01$). The length of hospitalizations was reduced ($t = 2.27$, df = 320, $p < 0.05$), but the rehospitalization rate increased ($\chi^2 = 82.93$, df = 1, $p < 0.01$), as well as the number of comorbidities ($\chi^2 = 11.19$, df = 1, $p < 0.05$). After 2013, the number of hospitalized PACG patients increased almost nine-fold ($\chi^2 = 40.83$, df = 1, $p < 0.01$), which is the main reason for the increase in the total number of PACG hospitalizations ($\chi^2 = 71.04$, df = 1, $p < 0.01$). During the same period, the average number of hospital days decreased from 7.2 to 5.5 days ($t = 1.94$, df = 70.93, $p = 0.05$), while the number of comorbidities increased.

The number of OG hospitalizations recorded from 2011 to 2013 increased significantly in the 2014–2017 period ($\chi^2 = 184.83$, df = 1, $p < 0.01$). The patients hospitalized after 2013 were on average four years older ($t = -3.12$, df = 498, $p < 0.05$). There was an increase in the number of hospitalized patients ($\chi^2 = 25.1$, df = 1, $p < 0.01$), but hospitalizations were shorter in duration ($t = 2.3$, df = 131.36, $p < 0.05$).

DISCUSSION

The average hospitalization rate of glaucoma patients in Kragujevac Clinical Centre in the 2006–2017 period was 4.9/10,000 inhabitants, while for Serbia the rate was lower – 2.8/10,000 inhabitants. This may be the result of the fact that the population of the Central and West Serbia regions gravitates towards Kragujevac Clinical Centre. In accordance with demographic changes, i.e. the increasing number of elderly people (age 65 and older) in the total population, the largest number of patients was registered in the geriatric population [12, 13].

During the given period, the total number of hospitalizations was fluctuating to a great extent, so we decided to divide this period into three time intervals: 2006–2010, 2011–2013, and 2014–2017. During 2006–2010, there was a mild decreasing trend in the number of glaucoma hospitalizations, while the number of rehospitalization increased almost three-fold. During the next time interval, both the number of hospitalizations and the number of rehospitalizations was almost halved. Since 2010, there was a significant increase in the number of rehospitalizations which contributed to an increase in the total number of hospitalizations. In 2006, the rate was one rehospitalization per seven hospitalizations, while 10 years later two rehospitalizations were registered for two hospitalizations. These results indicate that the number of newly diagnosed cases which require hospital treatment is decreasing.

Rehospitalized patients are actually chronic patients who do not manage to maintain intraocular pressure under control due to low compliance. This further suggests that there is a need for better education of patients, on one side, and more attention of doctors in the primary health care system, on the other.

In Central and West Serbia, the most common cases were OG glaucoma (44.6%), followed by POAG (37.9%) and PACG (17.5%). In Serbia in 2016, this order is the same, the most common being OG glaucoma (62.6%), followed by POAG (26.3%) and PACG (11.1%). In Central and West Serbia, POAG hospitalizations had a declining trend, while PACG and OG hospitalizations had increasing trends, more pronounced with PACG.

Similar research results were found in Africa and Asia. In Ethiopia, the most frequent cases are of exfoliate glaucoma (35.2%), POAG (32.8%), and PACG (18.5%) [14]. South-Central Asia is also projected to overtake East Asia in 2040 with the highest number of overall glaucoma cases and POAG burden, while PACG burden will remain the highest in East Asia [13].

The results have shown that the age of the patients increased regardless of the type of glaucoma, which is in accordance with the findings in other studies [14, 15, 16]. The sex analysis shows that women are at higher risk of PACG than men [17].

According to the experts' estimates with respect to population age, today in Serbia there are about 100 000 people with glaucoma and this number is expected to keep increasing. Unfortunately, there is no accurate data on the number of people with glaucoma since Serbia does not have an official register which should be introduced into the Law on Health Records, imposing an obligation to all institutions or healthcare professionals to conduct regular analyses. In the absence of such a registry, the data on the disease are partial since they are obtained from hospital records, disease history, reports of established illnesses and conditions, etc. American Academy of Ophthalmology Intelligent Research in Sight (IRIS) established this type of registry, being the first comprehensive clinical register of eye diseases. The Academy developed it as an integral part of the common goal of the profession to continuously improve the treatment of ophthalmologic diseases and glaucoma. Within this registry, visualization tools were developed, reports were prepared in the form of tables and charts (total number of diseases, demographic characteristics: by sex, age, ethnic group, type of glaucoma, disease progression, procedures, types of surgical interventions, health insurance) [18].

Holló et al. [19] as representatives of the European Glaucoma Association, pointed out that there are significant differences in the diagnosis, treatment and monitoring of glaucoma among European countries. The differences result from the different economic situations and the consequent financial ability to follow the recommendations and guidelines of the European Association of glaucomacologists [19, 20].

Studies indicate the importance of continuous education of patients, especially of the elderly and male population [21]. The analyses show that visual impairment caused by glaucoma affects the life quality of the patients, especially with less educated individuals [22, 23, 24]. The main goals of the glaucoma treatments are to preserve the visual functions of patients and to preserve/increase their life quality [25, 26, 27].

CONCLUSION

In Central and West Serbia there was a significant reduction of the length of hospital stay among patients with all glaucoma types during the researched time period. The hospitalization rates varied, but since 2013 there was a significant increase of hospitalizations which contributed to the overall increase of this rate. These findings can be attributed to inadequate prevention, untimely diagnosis, and inadequate treatment at the level of primary health care. Glaucoma is a disease of public health significance, because it is a major burden on society in terms of morbidity, disability, quality of life, as well as direct and indirect costs. Adequate secondary prevention measures, i.e. screening, can prevent the onset of the disease, shorten the length of hospitalization, reduce the number of rehospitalization, improve the outcome of treatment and prevent complications and loss of vision. The number of people with disabilities is expected to continue to rise due to inadequate prevention and the changes in demographic structure, i.e. the aging of the population and consequent exposure to more numerous risk factors.

Conflict of interest: None declared.

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Карактеристике хоспитализације болесника са глаукомом у Централној и Западној Србији

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САЖЕТАК

Увод/Циљ Глауком представља хронично обољење које узрокује иреверзибилне промене оптичког нерва које доводе до озбиљног оштећења вида и слепила. Инциденција примарног глаукома отвореног угла, као најчешћег облика глаукома у свету, на годишњем нивоу износи 2,4 милиона. Циљ рада је сагледати карактеристике хоспитализација узрокованих глаукомом у Централној и Западној Србији у периоду 2006–2017. године.

Методе Ова студија је ретроспективна анализа хоспитализација узрокованих глаукомом у Клиничком центру Крагујевац у периоду 2006–2017. године ($n = 1751$). Све хоспитализације подељене су према основном узроку болести у три подгрупе: примарни глауком отвореног угла, примарни глауком затвореног угла, секундарни и остали глаукоми.

Резултати Просечна стопа хоспитализације болесника са глаукомом износила је 5/10 000 становника. Најнижа стопа забележена је 2013. године (1,8/10 000), а највиша у 2015. години (9,3/10 000). Стопа рехоспитализација услед глаукома кретала се од 0,5/10 000 у 2013. години до 6,9/10 000 у 2015. години, просечно 2,4/10 000. Најзаступљенији су секундарни и остали глаукоми (44,6%,), затим примарни глаукоми отвореног угла (37,9%) и примарни глаукоми затвореног угла (17,5%). Дужина хоспитализације опада код свих врста глаукома са просечних 9,7 ± 6,5 (2006) на 5,5 ± 3,7 дана (2013).

Закључак У Централној и Западној Србији бележи се значајан пад дужине хоспитализације код свих подгрупа глаукома. Стопа хоспитализације болесника са глаукомом варира, са значајним порастом од 2013. године, што је последица пораста стопе рехоспитализације.

Кључне речи: примарни глауком отвореног угла; примарни глауком затвореног угла; секундарни глауком; хоспитализација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Structure of the attitudes towards cosmetic procedures' acceptance

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SUMMARY
Introduction/Objectives The objective of our study was to investigate the structure of the cosmetic procedures' acceptance attitudes and differences in acceptance between persons who had undergone minimally invasive cosmetic procedures and those who had not.
Methods The study included 245 subjects (treatment group), 21–73 years old (42.02 ± 12.12). The control group included 250 subjects who had not previously undergone cosmetic procedures, also 21–73 years old (40.19 ± 11.71). The control group was balanced with the treatment group according to category distribution of demographic variables. The Acceptance of Cosmetic Surgery Scale, adjusted for cosmetic procedures in general, was used for the evaluation of participants' attitudes towards these procedures.
Results Internal consistency of the scale was $\alpha = 0.963$, the split-half coefficient of validity was 0.861/0.810, and test–retest correlation coefficient was 0.892. The treatment group has shown overall higher acceptance ($t(478) = 27.024$, $p < 0.001$, $\eta^2 = 0.6$), and higher scores in all three dimensions. No demographic variable has shown significant differences in total or individual factor scores in either group.
Conclusion Subjects from both groups had scored higher on items dealing with the advantages of cosmetic procedures on a personal level (Intrapersonal Factor).
Keywords: minimally invasive cosmetic procedures; acceptance of cosmetic procedures; attitudes

INTRODUCTION

It is indisputable that there is a big and constant social pressure to achieve the physical appearance ideal in today's day and age, despite the constant changes of this ideal over the past 30 years [1]. The need to stay ever so young is also something that represents an important component of the modern era. Numerous researches have shown that people have a greater tendency to attribute positive personality traits to physically attractive individuals, who are then better treated in all aspects of everyday social interactions [2]. A similar tendency can be seen in social perception and reactions of observers to persons who have undergone certain aesthetic interventions. They evaluate these persons as younger, more attractive, more successful, and ascribe positive character traits to them [3]. Possibly the greatest advantage that is associated with better physical appearance (especially by people who perceive themselves negatively) are not social relationships, but rather their own psychological state, such as satisfaction with own body image and the quality of life [4].

Cosmetic procedures

The number of attempts at altering one's physical appearance through both surgical and non-surgical medical interventions is constantly rising [5, 6]. Nowadays, non-surgical and minimally invasive cosmetic procedures are increasingly popular, because they do not require much time, general anesthesia or major surgical interventions, they have short recovery periods, the patient can continue with everyday activities instantly, and the side-effects are minimal and relatively safe [7, 8]. Over the past 10 years, there was a significant improvement of non-invasive procedures, such as fillers, toxin injections, lasers and other technologies based on light, used for the rejuvenation of face, arms, breasts, as well as for the removal of aesthetic effects caused by aging, sun exposure, poor dietary habits, and smoking.

However, the specific trait of non-surgical cosmetic procedures is also the fact that they must be repeated over certain periods of time to maintain the desired appearance, which is why it is not unusual for one person to have more than five treatments over the course of a year [7, 8]. The most common non-invasive aesthetic procedures in both sexes are botulinum toxin injection, hyaluronic acid, hair

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transplantation, chemical peeling, and microdermabrasion [5, 6].

Acceptance of cosmetic procedures

Body image is a person's subjective perception of the aesthetics of their own body, and reflects their attitudes, thoughts, and emotions towards their own body, but also the way in which a person interprets the reactions of others. The modern era and advancement of aesthetic medicine impose social pressure towards one's physical appearance, and the fact that most people cannot achieve the physical appearance ideal makes them dissatisfied with their appearance. Despite that, not all people who are dissatisfied with their physical appearance choose to undertake medical aesthetic procedures. Factors that have so far been associated with the decision to undertake aesthetic medical procedures include intrapersonal factors such as unsatisfaction with personal appearance, appearance orientation, social factors – internalization of sociocultural messages, appearance conversations with peers, and pressure from the media for striving towards the physical appearance ideals [9, 10, 11]. Moreover, it was also found that positive experience with aesthetic procedures of people that are in close social proximity to a person (e.g. friend or family member) plays an important role [12].

Subjective component of cosmetic procedures' acceptance includes attitudes of a person towards the general physical appearance, or appearance of particular body parts [13]. The core aspect of discontent with own body image is a discrepancy between the perceived and ideal self, both in self-ideal, and the ideals imposed by society [8, 13]. Multiple studies have shown that people who decide to undertake cosmetic procedures have gone through ridicule or some other form of social pressure because of their physical appearance at a certain point in their lives. For instance, women who had undergone breast augmentation surgery reported a greater rate of appearance-related teasing than other women did [14].

The most common way of researching attitudes towards aesthetic procedures is investigating attitudes in the general population, with an emphasis on people's determination to undergo a certain cosmetic/aesthetic procedure, and the motivation that has led them to such a decision [15]. Two motivational factors emerge in that context – acceptance of cosmetic procedures for social and intrapersonal reasons, which is in line with the idea that one's physical appearance is reflected both through their self-image, as well as social impressions and interactions with others [15].

However, very few studies have investigated specific subpopulations that are not the general population.

This study is part of a larger study that has tried to find a connection between the acceptance of cosmetic procedures and certain personality traits. Nonetheless, as a first step and objective, it was necessary to examine and understand the basic characteristics, contents, and differences in attitudes towards cosmetic procedures between people who have undergone previous non-surgical, minimally invasive cosmetic procedures (the treatment group) and those who have not (the control group). The secondary objective was to validate (internal consistency, test–retest reliability, confirmative factor structure) the Acceptance of Cosmetic Surgery Scale in the population of people who had minimally invasive cosmetic procedures.

METHODS

The study was conducted at an aesthetic medical center in Belgrade, Serbia, over a period of three months. The study was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki–Tokyo). All the subjects filled out the questionnaire anonymously after they were informed about the purpose of the research.

General inclusion criteria were the subject's age of at least 18 years and signed informed consent form. Inclusion criteria for the treatment group was that the participant had undergone a non-surgical cosmetic procedure, and had not undergone any surgical aesthetic procedures. The control group was created from the general population, and adjusted with the treatment group for the distribution category in the demographic variables that were relevant to this study: sex, age, education, and marital status. Exclusion criteria in the control group were previous non-surgical or surgical cosmetic procedures.

Participants

The study included 245 participants in the treatment group, 13 (5.3%) male, and 232 (94.7%) female. The mean age was 42.02 ± 12.12 years, ranging 21–73 years. The study included 250 participants in the control group, 16 (6.4%) male, and 234 (93.6%) female. The mean age was 40.19 ± 11.71 years, ranging 21–73 years. Also, 100 participants from the control group were retested one month after the first test.

The control group was balanced with the treatment group in all basic demographic variables: sex ($\chi^2(1) = 0.107$, $p = 0.744$), age ($\chi^2(4) = 1.744$, $p = 0.783$), education ($\chi^2(3) = 5.931$, $p = 0.115$), marital status ($\chi^2(3) = 0.706$, $p = 0.872$), and the number of children ($\chi^2(4) = 2.436$, $p = 0.656$). The only demographic variables with statistically significant differences between the groups were employment status and economic status, with the participants from the treatment group having better employment status ($\chi^2(4) = 19.096$, $p < 0.001$), and economic status ($\chi^2(4) = 57.794$, $p < 0.001$).

A comparison of demographic variables was given in Table 1.

There were no statistically significant differences between the two groups in mean values of body mass index ($t(342) = -1.454$, $p = 0.147$).

Similar to previous studies conducted on the same population, the treatment group participants had on average 10 non-surgical cosmetic procedures, out of which the most frequent ones were facial (53%) [7, 8]. The most commonly

Table 1. Demographic characteristics of the subsamples

Treatment group									
Education	%	Marital status	%	Employment status	%	Economic status	%	No. of children	%
secondary	19.2	married	43.7	employed	79.2	lower middle	5.3	0	50.6
univ. students	7.3	with a partner	25.3	unemployed	7.8	middle	26.5	1	18
graduates	73.5	single	20.4	univ. students	4.9	upper middle	34.7	2	27.3
		other	10.6	pensioners	8.2	high	33.5	3 +	4.1
Control group									
Education	%	Marital status	%	Employment status	%	Economic status	%	No. of children	%
secondary	24.8	married	46	employed	66.8	lower middle	26.8	0	47.2
univ. students	10.4	with a partner	26.4	unemployed	16.4	middle	33.2	1	20
graduates	64.8	single	18	univ. students	9.6	upper middle	24	2	28
		other	9.6	pensioners	7.2	high	16	3 +	4.8

used techniques were different types of fillers and removal of stretch marks (around one quarter of the procedures, each), immediately followed by body mesotherapy (20%), face mesotherapy (16%), and hair removal, cavitation, etc. (up to 5%).

Measures

A questionnaire constructed for this study gathered information about the following sociodemographic characteristics of the participants: sex, age, education, economic status, employment status, marital status, number of children, and number of cosmetic procedures.

Acceptance of Cosmetic Surgery Scale is one of the most used instruments for assessment of attitudes towards cosmetic, aesthetic procedures, and has been standardized in multiple languages, including the Serbian version [9, 15, 16]. In our version, the instrument comprises the same 15 items that are answered through a seven-point Likert scale with answers ranging from 1 (I strongly disagree) to 7 (I strongly agree), with the only change being that the instructions emphasized that the term 'cosmetic procedure' includes not only surgical, but also minimally invasive cosmetic procedures (fillers, botulinum toxin, stretch marks removal, laser, etc.). In addition to the summed score, the scale can be divided into three factors: Intrapersonal, Social, and Consider. The first one measures attitudes related to self-oriented benefits of aesthetic procedures, enhancing self-esteem and personal satisfaction (e.g., "Cosmetic procedures are a good thing because they can help people feel better about themselves"). The second factor measures social motivation for having aesthetic procedures as a means of gaining social benefits or appearing more attractive to others (e.g., "I would seriously consider having cosmetic procedures if my partner thought it was a good idea"). The third factor measures the participants' interest for these procedures – the probability that a person would consider having an aesthetic procedure (e.g., "If I knew there would be no negative side effects or pain, I would like to try having a cosmetic procedure"). The scale showed high reliability and test–retest correlation [9, 15, 16]. It took 15–20 minutes to complete the questionnaire and the scale.

Statistical analysis

Besides descriptive statistics (central tendency measures and percentages), analyses for determining statistical differences were used: t-test for independent samples and ANOVA as well as χ^2 tests for categorical variables. For correlation analyses, we used Pearson's and Spearman's coefficients of correlation. Normal distribution was estimated by means of the Kolmogorov–Smirnov test. Confirmatory factor analysis, maximum likelihood method, was also used. The analyses were conducted in PASW Statistics, Version 18 (SPSS Inc., Chicago, IL, USA), as well as Amos 18 (Statistics Solutions, Clearwater, FL, USA).

RESULTS

Acceptance of cosmetic interventions

Internal consistency of the scale has shown high values of $\alpha = 0.963$, with item-total correlation (r) ranging 0.673–0.876. The split-half (Spearman–Brown) coefficient of validity was 0.861 in the control group, and 0.810 in the treatment group. Test–retest coefficient of correlation was $r = 0.892$.

The scale has shown good preliminary results that justified further factor analysis (Bartlett's test of sphericity $\chi^2(105) = 7454.35$, $p < 0.01$, KMO = 0.96). Based on existing research, we used confirmatory factor analysis, maximum likelihood method, and comparison of models with two or three factors (Table 2).

Table 2. Model fit indices

Model	χ^2/df	p	GFI	AGFI	CFI	RMSEA	PCLOSE
Two-factor model	3.320	< 0.001	0.932	0.897	0.975	0.069	0.001
Three-factor model	3.079	< 0.001	0.935	0.905	0.978	0.064	0.001

GFI – goodness of fit; AGFI – adjusted goodness of fit index; CFI – comparative fit index; RMSEA – root mean square error of approximation; PCLOSE – p of close fit

Due to the fact that preliminary analysis has shown that the two groups had differences in total scores on the scale, factor structure analysis was also conducted separately, by

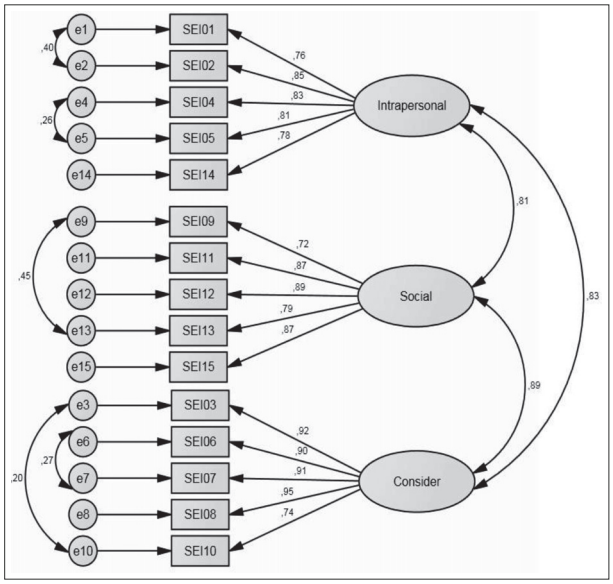

Figure 1. Three-factor model of the scale (for both groups)

groups. In the treatment group, the model accounts for a total of 67.04% variance, with the first factor accounting for 47.2%, the second factor for 10.77%, and the third factor for 9.08% variance.

In the control group, the model accounts for a total of 66.95% variance, with the first factor accounting for 48.41%, the second factor for 10.9%, and the third factor for 7.91%.

The items were distributed completely according to factors in both groups, and all had factor loadings greater than 0.5. Whereas the Social factor accounted for the least variance in the control group, this factor accounted for much more variance in the treatment group. In both groups, the Intrapersonal factor accounted for more variance than the Consideration factor.

Scores on factors show high intercorrelation, making it possible to analyze a unique, total score on the scale, which was possible on the original scale as well (Figure 1).

General descriptive data for factor scores and total scores on the Acceptance of Cosmetic Surgery Scale, according to groups, were given in Table 3.

Even though scores of both groups have a tendency towards higher values, these tendencies are more prominent in the treatment group, with the control group having milder tendencies, as shown on the histogram for one of the factors (Figure 2).

A comparison of group differences shows that the treatment group has higher total Acceptance ($t(478) = 27.024$, $p < 0.01$, $\eta^2 = 0.6$), as well as factor scores in Intrapersonal ($t(430) = 17.556$, $p < 0.01$, $\eta^2 = 0.38$), Consider ($t(441) = 27.218$, $p < 0.01$, $\eta^2 = 0.6$), and Social factors ($t(473) = 23.470$, $p < 0.01$, $\eta^2 = 0.53$), and all the differences had a large effect size.

The highest scores in the control group were given for items dealing with advantages of cosmetic, aesthetic procedures in general (e.g., "It makes sense to have a minor

Table 3. Descriptive statistics for acceptance factors

Factor	Group	Min–max.	Mean	Std. dev.	Std. error mean	Skewness	Kurtosis	z	α
Intrapersonal	treatment	5–35	31.18	4.728	0.302	-2.210	6.514	0.210**	0.85
	control	5–35	21.55	7.241	0.458	-0.400	-0.485	0.103**	0.85
Consider	treatment	5–35	30.95	5.493	0.351	-2.123	5.326	0.231**	0.88
	control	5–35	14.16	8.022	0.507	0.773	-0.426	0.137**	0.88
Social	treatment	5–35	24.29	7.449	0.476	-0.403	-0.612	0.075**	0.83
	control	5–33	9.84	6.176	0.391	1.825	3.052	0.217**	0.86
Acceptance	treatment	15–105	86.42	15.130	0.967	-1.307	2.518	0.120**	0.91
	control	15–103	45.55	18.393	1.163	0.692	0.209	0.074**	0.92

z – Kolmogorov–Smirnov
**p < 0.01

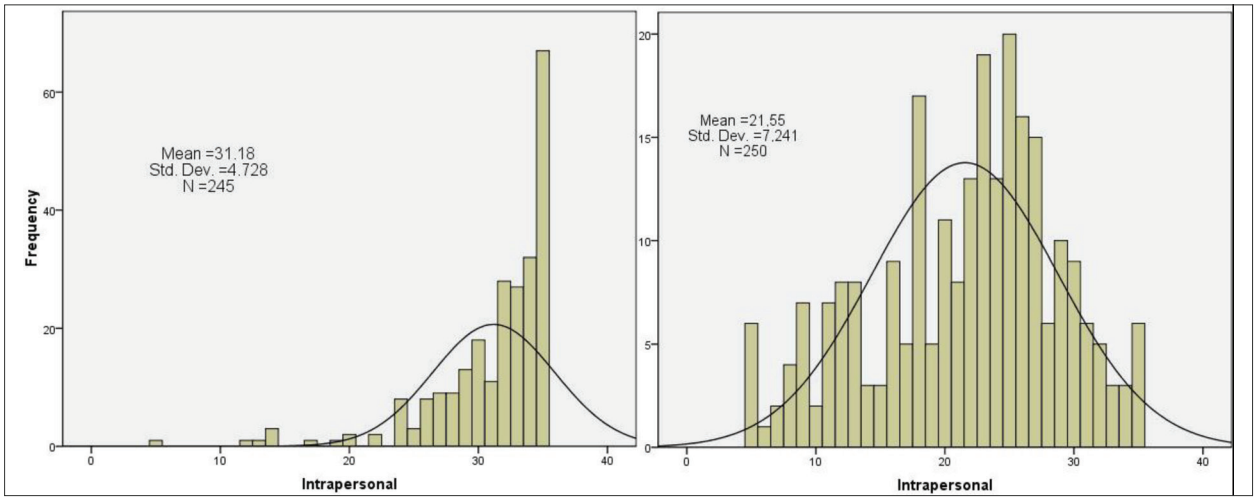

Figure 2. An example of the score distribution (Intrapersonal Factor)

Table 4. Differences in aesthetic intervention Acceptance (demographic variables)

Gender	Group	t	df	p
Intrapersonal	treatment	-1.779	243	0.076
	control	1.798	243	0.073
Consider	treatment	-1.528	243	0.128
	control	0.851	243	0.395
Social	treatment	0.735	243	0.464
	control	1.112	243	0.267
Acceptance	treatment	-0.744	243	0.458
	control	1.453	243	0.148
Education	Group	F	df	p
Intrapersonal	treatment	0.339	2, 246	0.713
	control	1.758	2, 246	0.175
Consider	treatment	1.808	3, 246	0.166
	control	0.187	3, 246	0.829
Social	treatment	0.352	3, 246	0.704
	control	0.498	3, 246	0.609
Acceptance	treatment	0.220	3, 246	0.803
	control	0.516	3, 246	0.598
Work status	Group	F	df	p
Intrapersonal	treatment	0.358	4, 245	0.783
	control	0.894	4, 245	0.468
Consider	treatment	0.288	4, 245	0.834
	control	0.220	4, 245	0.927
Social	treatment	0.383	4, 245	0.765
	control	0.376	4, 245	0.826
Acceptance	treatment	0.276	4, 245	0.842
	control	0.171	4, 245	0.953
Marital status	Group	F	df	p
Intrapersonal	treatment	0.936	2, 246	0.424
	control	0.853	2, 246	0.466
Consider	treatment	0.561	3, 246	0.641
	control	0.479	3, 246	0.698
Social	treatment	0.235	3, 246	0.872
	control	0.170	3, 246	0.917
Acceptance	treatment	0.487	3, 246	0.692
	control	0.242	3, 246	0.867

cosmetic intervention rather than spending years feeling bad about the way you look," M = 5.18, or "Cosmetic interventions are a good thing because they can help people feel better about themselves," M = 4.66). Whenever the items were about the issue of whether or not the participants themselves would actually try such a procedure, mean scores were lower. However, questions dealing with social acceptance, especially by the participant's partner had the lowest mean scores (e.g., "I would seriously consider having an aesthetic procedure if my partner thought it was a good idea," M = 1.72, or "I would seriously consider having a cosmetic procedure if I thought my partner would find me more attractive," M = 1.76).

The treatment group had the highest mean scores for items that belong to the Intrapersonal factor, i.e., are dealing with advantages of cosmetic procedures (e.g., "It makes sense to have a minor aesthetic procedure rather than spending years feeling bad about the way you look," M = 6.56, or "Aesthetic procedures are a good thing because they can help people feel better about themselves," M = 6.44). Similar to the control group, mean scores for items that were dealing with partner's social acceptance were the lowest (e.g., "I would seriously consider having an aesthetic procedure if my partner thought it was a good idea," M = 4.24), but much higher than in the control group.

Differences in acceptance according to demographic characteristics

Analyses in both groups have shown that no demographic characteristic showed significant differences in the total score or factor scores (Table 4).

Also, no correlation was found with continuous demographic variables (Table 5).

DISCUSSION

The three-factor model of cosmetic surgery acceptance that was created in this study, and that confirms the original structure of the questionnaire, comprised the following factors: Intrapersonal, Consider, and Social. The Intrapersonal factor accounted for most of the variance, followed by Social, and Consider factors.

It is obvious that the degree to which persons are satisfied or dissatisfied with their own body has strong implication for their self-awareness, self-respect, and social behavior, as well as their attitudes towards acceptance of cosmetic procedures. Body image dissatisfaction impacts the quality of life, and it is believed to be a motivation for a number of body altering procedures as well as related activities (being on diets, getting informed about cosmetic procedures, saving money, medical tourism, and the like) [17, 18]. This is further supported in our study by high scores for items such as, "It makes sense to

Table 5. Correlation coefficients between Acceptance factors and demographic variables

Variable	Group	Intrapersonal	Consider	Social	Acceptance
Age	treatment	r = 0.015, p = 0.810	r = -0.075, p = 0.242	r = -0.073, p = 0.254	r = -0.058, p = 0.362
	control	r = 0.100, p = 0.116	r = -0.056, p = 0.378	r = 0.050, p = 0.436	r = 0.031, p = 0.621
Body mass index	treatment	r = -0.092, p = 0.215	r = -0.087, p = 0.239	r = -0.053, p = 0.471	r = -0.087, p = 0.241
	control	r = -0.020, p = 0.804	r = -0.089, p = 0.264	r = -0.040, p = 0.622	r = -0.062, p = 0.438
Number of children	treatment	r = 0.060, p = 0.347	r = 0.024, p = 0.706	r = 0.084, p = 0.190	r = 0.069, p = 0.282
	control	r = 0.105, p = 0.100	r = 0.000, p = 0.996	r = 0.052, p = 0.414	r = 0.059, p = 0.357
Economic status	treatment	r = 0.074, p = 0.246	r = 0.083, p = 0.198	r = 0.054, p = 0.397	r = 0.080, p = 0.212
	control	r = 0.054, p = 0.398	r = 0.008, p = 0.903	r = -0.002, p = 0.969	r = 0.024, p = 0.711

have a minor aesthetic intervention rather than spending years feeling bad about the way you look," or "Cosmetic interventions are a good thing because they can help people feel better about themselves." Other studies have also found that sociocultural influences are not the only significant factor for the development of attitudes towards cosmetic interventions, but rather that specific aspects of the self also play an important role. For instance, self-monitoring and self-awareness (both private and public) had a direct effect on women's consideration of breast cosmetic surgery [19].

Other studies have also shown that the feelings about one's own looks are the key factor in deciding upon a cosmetic surgery, and that reactions to changes caused by these interventions are more positive if reasons are personal and not under the influence of the partner [20]. On the other hand, even though our participants had lowest mean scores for items dealing with social factors, especially partner opinions, high pressure on a person is certainly being made by various social and cultural influences. Despite the possibility that people consciously reject the importance of influence of other people, negative comments about someone's physical appearance may be interpreted as subconscious pressure, thus causing dissatisfaction and low self-esteem [8]. Furthermore, imposed social standards and ideals of physical appearance that are broadcasted through media, especially by fashion and aesthetic industry advertisements, have huge influence on our own body image perception, and may become internalized standards for understanding the importance of physical appearance [11, 21]. Additionally, as the popularity of aesthetic procedures grows, so does the media attention and general social acceptance of cosmetic procedures – therefore, the general interest for aesthetic procedures also grows.

The risk of developing dissatisfaction with one's own body image is higher in persons who constantly compare themselves with others [4, 22]. Prior studies which have used this scale have shown that the lower a person's appearance and social self-esteem is, the more likely she/he is to accept cosmetic surgery [15]. In line with that, a particular score stood out on the Social factor, negatively correlating with social self-esteem. The fact that most people, more often women, decide to undergo aesthetic procedures after they are 35 and in their early 50s, i.e. at the first sign of old age and in the climacteric period, is unsurprising [7]. It is also not unusual how acceptance of cosmetic procedures is linked with lower self-esteem and self-confidence, as well as tendencies towards hyperthymic temperament and conformity [8, 22].

Finally, this type of factor categorization may correlate with the principles of the theory of planned behavior, because the final decision (in our case the Consider factor) stands under the influence of Intrapersonal factors, beliefs and prior experience, and Social factors, pressures, and norms in the social environment of the person [23].

Differences according to demographic characteristics

Compared to the original study, our study had no connections between the sex and the age of the participants (in either group) and scores on this scale [15]. It is essential to underline that the fact that there are prominent differences between the two groups in acceptance (particularly on the social factor) but no differences according to demographic variables, speaks in favor of other personal characteristics, most likely of psychological and/or social nature, which have a greater impact on accepting this type of an intervention. Therefore, in future studies, researchers should examine the influence of some of the psychological characteristics, ideally through a longitudinal study.

The scale characteristics

It should also be noted that the Serbian version of this scale has been adapted for cosmetic procedures in general (not only surgical procedures), and has exhibited good overall internal consistency and construct validity scores in both groups. The test–retest reliability coefficient indicates a stable reliability over time. Our study also corroborates evidence from previous studies reporting that the three factors are mutually dependent, and one total score can be used when describing cosmetic intervention acceptance.

CONCLUSION

The feelings about one's own looks are the key factor in deciding upon a cosmetic surgery. However, even though our participants had the lowest mean scores for items dealing with social factors, high pressure on a person's body image perception is certainly being made by various social and cultural influences. Finally, the Serbian version of this scale has been adapted for cosmetic procedures in general (not only surgical procedures) and has exhibited good psychometric properties.

Conflict of interest: None declared.

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Структура ставова према прихватању естетских интервенција

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САЖЕТАК

Увод/Циљ Циљ студије је био испитати структуру ставова према прихватању естетских интервенција и могуће разлике између особа које су претходно имале неку минимално инвазивну естетску интервенцију и оних који то нису.

Методе У студију је укључено 245 испитаника (третирана група), од 21 до 73 године старости (42,02 ± 12,12). Контролну групу је чинило 250 испитаника који нису имали претходне естетске интервенције, такође од 21 до 73 године старости (40,19 ± 11,71). Контролна група је избалансирана у односу на дистрибуцију одговарајућих демографских категорија испитаника из третиране групе. За процену ставова испитаника према естетским интервенцијама коришћена је Скала прихватања естетских хируршких интервенција, прилагођена за све врсте ових процедура.

Резултати Интерна конзистентност скале износила је $\alpha = 0{,}963$, коефицијент валидности *split-half* 0,861/0,810, а коефицијент корелације тест–ретест је износио 0,892. Третирана група је бележила значајно веће прихватање ($t(478) = 27{,}024$, $p < 0{,}001$, $\eta^2 = 0{,}6$) и више бодовне резултате на све три димензије скале. Категорије демографских варијабли, у обе групе, нису показале значајне разлике у укупним и факторским резултатима на скали.

Закључак Испитаници из обе групе су постигли више бодовне резултате на тврдњама које се односе на предности естетских интервенција из интерперсоналних разлога.

Кључне речи: минимално инвазивне естетске процедуре; прихватање естетских интервенција; ставови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The importance of 6-MAM levels and morphine/codeine ratio in diagnosis of death among drug addicts

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SUMMARY

Introduction/Objective Heroin is metabolized to 6-monoacetylmorphine (6-MAM) and morphine. The objective of this study is to examine 6-MAM, morphine, and codeine relationships in order to distinguish deaths related to heroin consumption from deaths related to morphine and/or codeine consumption.

Methods The autopsy blood and urine samples from 45 opioid drug addicts were examined. Gas chromatography/mass spectrometry was applied to evaluate morphine, 6-MAM, and codeine. Two groups were formed: 6-MAM-positive (n = 35) and 6-MAM-negative (n = 10).

Results Compared to the 6-MAM-negative group, blood morphine levels were higher in the 6-MAM-positive group ($p = 0.022$), while blood codeine levels were similar ($p = 0.575$). In the 6-MAM-negative group, the blood morphine/codeine ratio was 8.3, and it was 4.3 in the 6-MAM-positive group. There was no difference between the groups regarding urine morphine levels ($p = 0.859$). The urine morphine/codeine ratio was 6.2 in the 6-MAM-negative group, whilst it was 32.2 in the 6-MAM-positive group. In the blood samples, morphine and codeine concentrations were significantly correlated ($r = 0.607$; $p = 0.006$). In urine samples, correlations between morphine and codeine ($r = 0.766$; $p < 0.001$), morphine and 6-MAM ($r = 0.650$; $p < 0.001$), as well as codeine and 6-MAM ($r = 0.620$; $p < 0.001$), were also significant.

Conclusion Analyses of 6-MAM and morphine/codeine ratio in blood and urine autopsy samples may be used as diagnostic tools to distinguish deaths related to the consumption of different opioid drugs.

Keywords: autopsy; heroin; 6-MAM; morphine; codeine

INTRODUCTION

Heroin (3,6-diacetylmorphine) is an opioid drug synthesized by acetylation of morphine derived from crude opium. Beside morphine, the crude opium contains several other alkaloids, including codeine (3-methoxymorphine). During morphine synthesis, codeine also undergoes acetylation reaction yielding 6-acetylcodeine. As the result, heroin often contains a certain quantity of codeine as an impurity.

Heroin and codeine are actually prodrugs, which are both metabolized into the common active form – morphine. Heroin is metabolized into morphine via several intermediary products. After an intravenous injection, heroin is within three minutes converted into 6-monoacetylmorphine (6-MAM) by hydrolysis of one acetyl group. Thereafter, 6-MAM is metabolized into morphine during a time frame between 20 minutes and three hours [1, 2]. Codeine is converted into morphine by o-demethylation via hepatic cytochrome P450-containing enzyme – CYP2D6 [3]. Compared to heroin metabolism to morphine, the conversion of codeine into morphine is a relatively slow process, and in codeine intoxication it may take 24 hours before measurable morphine levels appear in the blood [4].

Positive detection and measurement of heroin metabolites in autopsy body fluids, without previous knowledge of death circumstances, can be ascribed to heroin consumption, but also to morphine intoxication which may occur during medical treatment of acute and chronic severe pain. According to the literature data, there are two indicators of heroin exposure in living individuals: the presence of 6-MAM in body fluids and tissues, and morphine/codeine ratio > 1. The latter is considered to be

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especially important in cases where 6-MAM was not detected, as well as in cases suspected for codeine intoxication [5, 6, 7]. However, these relationships seem to be by far the most complex in forensic autopsy samples, and, with few exceptions, poorly investigated in Serbian drug addicts.

This study evaluated blood and urine toxicological indicators of heroin use, 6-MAM and morphine/codeine ratio, obtained during medicolegal death investigation of cases suspected of heroin or morphine intoxication, in order to distinguish deaths related to heroin consumption from those related to the consumption of morphine and/or codeine.

METHODS

This study was performed at the Milovan Milovanović Institute for Forensic Medicine, Faculty of Medicine, University of Belgrade, Serbia, after ethical institutional clearance was obtained. From January 2006 to December 2015, a total of 12,817 autopsies were done, of which 351 autopsy cases of adult drug addicts, of both sexes. The inclusion criteria in the study were as follows: (1) death due to suspected accidental heroin intoxication, and (2) in addition to morphine, the presence of codeine and/or 6-MAM in autopsy blood and urine samples. Forensic autopsy files, heteroanamnestic data from the closest relatives, police records, and medical history files for all the studied cases were also collected. Of all autopsies performed in that period, 219 were associated with psychoactive substances abuse, but only 134 cases satisfied the inclusion criteria.

The postmortem blood and urine samples were taken with sterile syringe into chemically clean glass tubes. Approximately 50–100 mL of blood was taken from the femoral vein, and about 50–100 mL of urine was taken through the intact urine bladder wall. The samples were thereafter aliquoted and kept in closed tubes at -20°C for no longer than four weeks before analyzing.

Morphine, codeine, and 6-MAM were screened and quantitatively analyzed by gas chromatography (GC) with mass spectrometry (MS) detection, using a headspace technique, adopted for opiates [8]. We used Agilent 7000 GC/MS triple quadrupole gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA), connected to the flame ionization MS detector. Opiates were extracted from specimens according to instructions, using a capillary extraction column Oasis MCX-3cc (Waters Oasis®, Waters Corporation, Milford, MA, USA) [8]. Free morphine and codeine were screened in the 40–500 range, and a specific m/z ratio was used for the detection of a target ion. All positive specimens were further analyzed according to the manufacturer's instructions, using the selective ion monitoring mode [9]. Morphine was determined at m/z 429 (retention time: 11.6 min.), codeine at m/z 371 (retention time: 11.19 min.), and 6-MAM at m/z 399 (retention time: 12.47 min.). Concentrations of morphine, codeine, and 6-MAM were calculated from correspondent standard curves.

The data were processed using the statistical program package SPSS, version 22 (IBM Corp., Armonk, NY, USA). The data was presented according to standard descriptive statistics. Discontinual variables were expressed as frequencies and continual variables as median values (and range). Differences between the groups were tested by the Mann–Whitney U-test. Correlation analysis was accomplished by calculating Spearman's coefficient. Findings were considered statistically significant at $p < 0.05$.

RESULTS

By screening of autopsy samples from 219 death cases associated with the use of psychoactive substances, the detection of morphine alone occurred in 134 cases (63%), and in 63 other cases (28.8%) morphine was combined with another psychoactive substance, most frequently with codeine (56 cases; 25.6%). The results are presented in Table 1.

Samples where morphine alone was detected (n = 134) were further quantitatively analyzed. Beside morphine, detectable quantities of codeine and/or 6-MAM were found in 49 cases. The morphine/codeine concentration ratio (M/C) was calculated. In 45 cases, the M/C ratio in the blood and/or urine was > 1, and four urine samples had M/C ratio < 1. Of 45 cases with M/C ratio > 1, measurable levels of 6-MAM in body fluids were found in 35 cases (77.8%), and those comprised the 6-MAM-positive group. In other cases (n = 10), no measurable 6-MAM was detected, and these comprised the 6-MAM-negative group. The results are presented in Tables 2 and 3.

Median values of blood morphine and codeine, and urine morphine and codeine concentrations in 6-MAM-positive and 6-MAM-negative groups are presented in Table 4. As presented, blood morphine levels significantly differed between the groups and were approximately two times higher in the 6-MAM-negative than in the 6-MAM-positive group ($U = 3.5$; $p = 0.022$). Concentrations of blood codeine were similar in both groups ($U = 19.0$; $p = 0.575$). However, in the 6-MAM-negative group, the median blood M/C ratio was 8.25 and in the 6-MAM-positive group the median blood M/C ratio was 4.25.

Compared to the 6-MAM-negative group, median values of urine morphine levels were somewhat higher in the 6-MAM-positive group (Table 4), but the difference between the groups was not significant ($U = 134.0$; $p = 0.859$). Compared to the 6-MAM-negative group, the medium urine codeine concentrations were about three times lower in the 6-MAM-positive group. The differences between the groups regarding urine codeine levels were also not significant ($U = 129.0$; $p = 0.734$). However, in the 6-MAM-negative group, the median urine M/C ratio was 6.2, whilst in the 6-MAM-positive group, the median urine M/C ratio was 32.2, and this difference was statistically significant ($p < 0.001$).

We further analyzed relationships between morphine, codeine, and 6-MAM within each body fluid compartment. The results are presented in Table 5. In the current study there were significant correlations between blood morphine and blood codeine levels ($r = 0.607$; $p = 0.006$), urine morphine and urine codeine levels ($r = 0.766$;

Table 1. Opiates and their combinations in drug addicts who died from abuse of psychoactive substances from January 2006 to December 2015

Psychoactive substance(s)	Number of cases (n)	%
Morphine	134	63
Codeine	2	0.9
Methadone	4	1.8
Others	1	0.4
Morphine + codeine	56	25.6
Morphine + methadone	2	0.9
Morphine + tramadol	2	0.9
Morphine + methadone + tramadol	1	0.5
Morphine + codeine + tramadol	1	0.5
Morphine + codeine + methadone	1	0.5
Unknown	11	5
Total	219	100

Table 2. Distribution of opioid addict cases according to the presence of 6-MAM

Presence of 6-MAM	Number of cases (n)	%
6-MAM-negative	10	22.2
6-MAM-positive	35	77.8
Total	45	100

Table 3. Distribution of opioid addict cases with M/C ratio > 1, according to the presence of 6-MAM in the blood and urine

Presence of 6-MAM	Body fluid			
	Blood		Urine	
	n	%	n	%
6-MAM-negative	3	15.8	9	22.5
6-MAM-positive	16	84.2	31	77.5
Total	19	100	40	100

Table 4. Morphine and codeine concentrations in postmortem body fluids according to the presence of 6-MAM

Opioids in body fluids (μg/ml)	6-MAM n body fluids			
	6-MAM-negative		6-MAM-positive	
	n	Median (range)	n	Median (range)
Morphine (blood)	3	0.33 (0.23–0.39)	16	0.17 (0.03–0.41)
Morphine (urine)	9	2.37 (0.03–17.20)	31	3.33 (0.10–44.28)
Codeine (blood)	3	0.04 (0.02–0.11)	16	0.04 (0.004–0.090)
Codeine (urine)	9	0.38 (0.01–4.93)	32	0.11 (0.01–8.65)

Table 5. Correlation analysis for relationships between morphine, codeine, and 6-MAM within each body fluid compartment

Drug	Body fluid		Codeine (μg/mL)		6-MAM (μg/mL)	
			Blood	Urine	Blood	Urine
Morphine (μg/mL)	Blood	r	0.607		-0.162	
		p	0.006*		0.580	
		n	19		14	
	Urine	r		0.766		0.650
		p		< 0.001*		< 0.001*
		n		40		29
Codeine (μg/mL)	Blood	r			0.114	
		p			0.699	
		n			14	
	Urine	r				0.620
		p				< 0.001*
		n				28

Spearman's correlation coefficient (r) was calculated, and significant relationships were marked (*).

p < 0.001), urine morphine and urine 6-MAM levels (r = 0.650; p < 0.001), and between urine codeine and urine 6-MAM levels (r = 0.620; p < 0.001).

DISCUSSION

The M/C ratio is now considered to be a useful parameter to distinguish heroin-associated death from death associated with codeine abuse, since in the latter cases the M/C ratio is often ≤ 1 [7, 10]. Beside morphine, the presence of codeine and/or 6-MAM was detected in 49 of 134 cases (36.5%) in the current study. In 45 cases, the M/C ratio in the blood and/or urine was > 1, and in four cases the urine M/C ratio was < 1. Of these 45 morphine- and codeine-positive cases, in 35 cases (77.8%) we also detected measurable amounts of 6-MAM in the blood and/or urine samples. The M/C ratio in morphine- and codeine-positive cases varied 2.5–13. Previously, the M/C ratio was determined exclusively in the blood samples [5, 11, 12], and more recently the M/C ratio was calculated from both blood and urine samples [4].

For identification and measurement of 6-MAM, as currently most accurate molecular fingerprint of heroin exposure, it is often recommended to employ urine samples. The main reason is that, in contrast to the blood, 6-MAM once excreted into the urine is not further metabolized in organism [13, 14]. In Norway, Konstantinova et al. [4] investigated the M/C ratio in a large number of cases (n = 2,438), positive for 6-MAM and/or morphine and codeine in the blood and urine samples. In that study, in 98% of blood samples and in 96% of urine samples, the M/C ratio was > 1, and varied 5–10, which is largely in accordance with our findings. Moreover, one Swedish study of 747 cases related to heroin abuse also reported that in 98.9% of 6-MAM-positive cases the M/C ratio was > 1, although the M/C ratio was actually very high, with median value found to be 11 [6]. Estimating the survival times in acute heroin intoxication, Darke and Duflou [1] reported 6-MAM in 43% of the cases. This study also reported that 6-MAM-positive cases have two times higher morphine levels than 6-MAM-negative cases, which was also found in the current report (Table 4). Ceder and Jones [5] evaluated 6-MAM, morphine, codeine, and M/C ratio in living persons imprisoned for driving under the influence of drugs. Of 675 blood samples in that study, 6-MAM was present in 16 (2.3%), and of 339 urine samples, 6-MAM was recorded in 212 samples (62%). When 6-MAM was detected in urine, the blood M/C ratio was always > 1, and varied 1–66, with a median value of 6 [5].

However, the M/C ratio should be analyzed through the prism of free morphine and codeine levels. In the current study, we observed high median levels of blood morphine in both 6-MAM-positive (0.17 mg/L) and 6-MAM-negative cases (0.33 mg/L), and even higher median levels of urine morphine in 6-MAM-positive (3.33 mg/L) and 6-MAM-negative cases (2.37 mg/L). In contrast to that, median values of either blood or urine codeine levels were much lower and were not related to the 6-MAM status (0.04 mg/L, and 0.11 mg/L, respectively). These results are in accordance with several other studies who reported

that blood free morphine levels vary 0.22–0.60 mg/L and blood free codeine levels vary 0.01–0.07 mg/L after heroin exposure [4, 6, 7, 8].

We observed that, compared to the 6-MAM-negative group, concentration of the blood free morphine was lower in the 6-MAM-positive group (0.33 mg/L *vs*. 0.17 mg/L). Although this is opposite to several other studies which reported higher blood morphine levels in the6-MAM-positive group [4], our findings are in accordance with Ceder and Jones [5], who also found higher median values of blood morphine in the 6-MAM-negative group.

Also, morphine levels were higher than codeine levels in all 6-MAM-positive cases and all 6-MAM-negative cases with M/C ratio > 1, either in the blood or urine samples (Table 4). Moreover, blood morphine and blood codeine levels, as well as urine morphine and urine codeine levels, were significantly correlated (Table 5). These findings are somewhat expected, and may be partly explained by the fact that illegal production of heroin from crude opium is necessarily accompanied by the increased presence of codeine, as an impurity, thus heroin drug addicts consume some codeine as well. In the 6-MAM-positive group, there were statistically significant correlations between 6-MAM and morphine, as well as between 6-MAM and codeine in urine samples. Similar results were reported on cadaveric samples, as well as on samples from living drug addicts, thereby confirming that morphine and codeine levels in the body fluids are tightly related to the quantity of heroin taken with the dose [4, 5, 7].

In four 6-MAM-negative cases the blood M/C ratio was > 1, whilst the urine M/C ratio was < 1, with a median of 0.55. Konstantinova et al. [4] also reported low values of M/C ratio in 2% of blood and 4% of urine samples but among 6-MAM-positive cases, while Ceder and Jones [5] reported M/C ratio < 1 in 15% cases of 6-MAM-positive living, imprisoned drug addicts. One probable explanation of such divergent M/C ratio findings observed among 6-MAM negative cases could be related to longer survival time, during which the blood 6-MAM could undergo further metabolic reactions yielding products that were not targeted in our study.

Codeine is an opioid analgesic drug. However, median concentration of free codeine in four cases with urine M/C ratio < 1 in our study was 0.67 mg/M (0.31–1.10 mg/L). This was 2.4 times higher than free morphine levels in the same group (median 0.28 mg/L; range: 0.024–0.94 mg/L), and several times higher than therapeutic codeine dose. Pharmacokinetic studies have well documented that the blood concentration of free codeine is several times higher than free morphine after one or more codeine doses [9, 15, 16]. Kronstrand et al. [17] reported, for example, that ingestion of 100 mg codeine phosphate gave rise to the free blood codeine to 0.183 mg/L, while in the study by Quiding et al. [13], ingestion of 60 mg codeine phosphate gave rise to blood codeine of 0.115 mg/L. Ceder and Jones [5] reported blood free codeine levels of 0.180 mg/L in living drug addicts with M/C ratio < 1. These and our results suggest that a low M/C ratio probably indicates that codeine was deliberately used some time before death, or, more likely, that codeine as an impurity was highly present in the heroin dose.

CONCLUSION

The current study of death related to chronic heroin exposure indicates that the presence of 6-MAM and M/C ratio in body fluids may be used as reliable tools to differentiate cases in which it is unclear whether death resulted from (chronic) heroin and morphine consumption for non-medical (recreational) purposes, or from accidental therapeutic use of morphine.

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Значај концентрације 6-MAM и односа морфин/кодеин у дијагностици смрти наркомана

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САЖЕТАК

Увод/Циљ Хероин се у организму метаболише у 6-моноацетилморфин (6-MAM) и морфин. Циљ рада је да испитујући концентрације 6-MAM, морфина, кодеина и њихових односа диференцијално дијагностички дефинишемо разлику између смрти које су наступиле уношењем хероина у организам од оних које су наступиле коришћењем морфина и или/кодеина.

Методе Код 45 обдукованих наркомана код којих је у узорцима крви и урина однос вредности концентрација морфин/кодеин > 1, гасном хроматографијом / масеном спектрометријом одређиване су концентрације морфина, 6-MAM-а и кодеина. Формиране су две групе испитаника: 6-MAM позитивна ($n = 35$) и 6-MAM негативна група ($n = 10$).

Резултати У поређењу са 6-MAM негативном групом, концентрација морфина у крви је била значајно већа у 6-MAM позитивној групи ($p = 0,022$), док се вредности кодеина у крви нису разликовале ($p = 0,575$). У 6-MAM негативној групи однос морфин/кодеин у крви је био 8,3, док је у 6-MAM позитивној био 4,3. Није било значајне разлике у концентрацији морфина у урину међу групама ($p = 0,859$). Однос морфин/кодеин у урину у 6-MAM негативној групи је био 6,2, док је у 6-MAM позитивној групи био 32,2. Концентрације морфина и кодеина у крви су значајно корелирале ($r = 0,607$; $p = 0,006$), као и концентрације морфина и кодеина у урину ($r = 0,766$; $p < 0,001$), морфина и 6-MAM у урину ($r = 0,650$; $p < 0,001$) и кодеина и 6-MAM у урину ($r = 0,620$; $p < 0,001$).

Закључак Присуство 6-MAM у узорцима телесних течности и однос морфин/кодеин могу бити корисни индикатори и послужити у дијагностици смрти повезаној са узимањем опијата.

Кључне речи: обдукција; хероин; 6-MAM; морфин; кодеин

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Human dirofilariasis

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SUMMARY

Introduction Dirofilariasis is a zoonosis caused by nematodes from the genus *Dirofilaria*, which is a parasite in dogs and other canids, and humans get infected by a mosquito bite. In Europe, the number of patients outside the endemic area is increasing. So far, more than 2,850 cases of human dirofilariasis have been reported worldwide. In the last 20 years, we have had only two confirmed cases in our institution. The disease is manifested in a cutaneous, visceral, and ocular form. From the initial infection, the first symptoms can take several years to manifest. The diagnosis can be confirmed histologically, morphologically, and/or by molecular techniques. The treatment includes surgical removal of the parasite and antiparasitic therapy.

Case outline The paper presents two cases of *Dirophilaria repens* infection. The first patient had a migratory nodular facial skin change for several years. After the skin induration incision in the zygomatic region, a 7-cm-long worm was extracted, later identified as *Dirophilaria repens*. The pathohistological finding of the extirpated change showed that it was a granuloma inflammation. The second case was a patient with a persistent cough and hemoptysis, with a morphologically verified nodular change in the pulmonary parenchyma. The pathohistological finding of the extirpated change showed a chronic granulomatous inflammation and the presence of parasites. The treatment of both patients resulted in a complete recovery without complications.

Conclusion In case of subcutaneous nodules or unclear lung changes, dirofilariasis should be considered. Video-assisted thoracoscopic surgery is the leading diagnostic surgical procedure concerning dirofilariasis, and a significant therapeutic modality.

Keywords: dirofilariasis; parasitosis; video-assisted thoracoscopic surgery

INTRODUCTION

Dirofilariasis is a zoonosis caused by nematodes from the genus *Dirofilaria*. The parasite is transmitted to humans from infected animals, most often dogs, accidentally, by an infected mosquito bite. The infection includes only one worm, and it may take several decades until the first symptoms occur. The disease is benign and very rare outside the endemic area. In Europe, the number of patients has been steadily increasing for the last ten years [1]. There are three forms of the disease, subcutaneous, visceral and ocular.

By the year 2012, there were 1,782 cases of human dirofilariasis reported, out of which 1,410 in Europe, and 372 in the USA [2]. In Serbia, 37 cases were reported by 2014 [3]. The diagnosis is based on anamnestic data, clinical course, possible parasite visualization, surgical extirpation, pathohistological verification, and, rarely, serological diagnosis. The treatment can be surgical and conservative. Prophylaxis includes the treatment and prophylaxis in dogs as a reservoir, the suppression of mosquitoes as vectors, and the protection of humans by means of repellents.

CASE REPORT

This paper presents two cases of *Dirofilaria repens* infection, which were confirmed at the Clinic for Infectious and Tropical Diseases of the Military Medical Academy in the last 20 years.

The first case was diagnosed in 1998 in a woman aged 64 from the village of Kusadak, located in the vicinity of Belgrade. She had had a migratory nodular facial skin change for several years. Since her youth, the patient had been having a dry, irritating cough, and later some problems in the form of redness and itching of the skin. Three years prior to diagnosis, some migratory tumor changes occurred on the scalp, and then she developed swelling and redness of the upper eyelid region. Further on, pain, redness and skin induration of the right zygomatic region occurred. When pressure was applied to the induration, a vital 7-cm-long parasite was extracted (Figure 1). The identification was carried out at the Institute of Medical Biochemistry of the Military Medical Academy where *D. Repens* female was confirmed. The patient's clinical findings were dominated by a zygomatic bone swelling, together with induration and hyperemia. All laboratory findings were within reference values, while serological analyses were negative. Histopathological examination of the extirpated change showed granuloma inflammation.

The second case was diagnosed in 2018 in a 72-year-old male from Belgrade. He first came to the doctor's due to persistent cough and hemoptysis. Morphological tests and multislice

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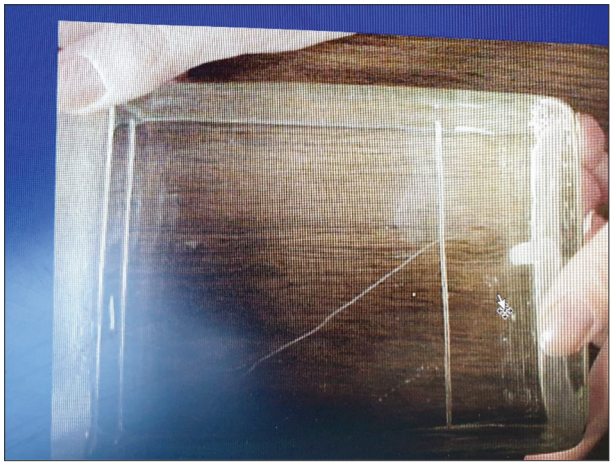

Figure 1. Extracted vital 7-cm-long parasite

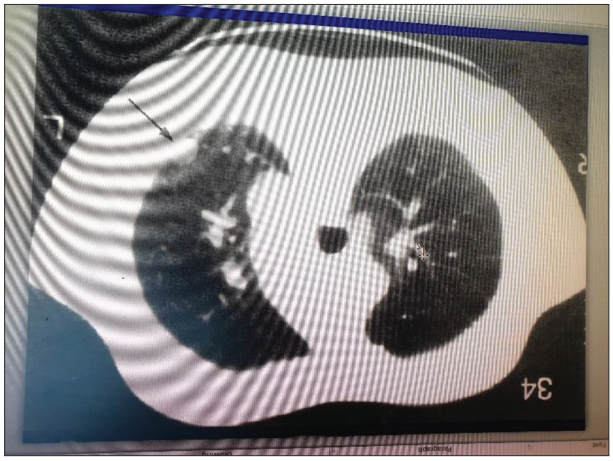

Figure 2. Multislice computed tomography of the thorax showed a lobulated, infiltrative change 16 × 16 × 11 mm in segment VI of the right lower lung lobe

computed tomography (MSCT) of the thorax showed a lobulated, infiltrative change 16 × 16 × 11 mm in segment VI of the right lower lung lobe, showing signs of infiltration of the surrounding parenchyma, followed by minimal pneumonitis (Figure 2). Video-assisted thoracoscopic surgery (VATS) was performed and a knot tissue sample 27 × 15 mm in size was obtained. Histopathological examination showed chronic granulomatous inflammation with necrosis similar to dirofilariasis. The patient was feeling well all the time and all his laboratory parameters were within reference values. Albendazole therapy was a used for 28 days. In the further course of the disease, the patient lost all subjective symptoms, while the definitive suspicion of a malignant neoplasm was removed by the control MSCT thorax examination.

DISCUSSION

Natural hosts of *Dirofilaria* are dogs and wild canids, such as foxes, wolves, and raccoons. Humans get infected by a mosquito bite. In Serbia, every species of mosquitos transmits the parasite [4]. The infection in humans is most commonly caused by three species: *D. immitis*, *D. repens*, and *D. tenius*. *Dirofilaria immitis* usually causes human pulmonary dirofilariasis throughout the world, while subcutaneous dirofilariasis caused by *D. repens* is recorded in Europe [5]. These two species are able to cause both pulmonary and extrapulmonary infection. It most commonly occurs in adults between the ages of 21 and 60 years (ESDA), but the case of a 14-month-old child with *Dirofilaria* in the scrotum region has also been described, which is its most common localization in children [6]. Women are more likely to be infected than men, but without a significant statistical difference. Generally, it occurs rarely in people, has a benign character, and, in most cases, the diagnosis is made by histopathological examination, and extremely rarely, as in our first case, by the evacuation of a live parasite.

The endemic areas for dirofilariasis are Asia, Africa, the Mediterranean, but in the past decade, there has been an increasing number of cases reported outside the endemic area, i.e. in the region of northern and central Europe [1]. The highest percentage of cases was registered in Italy (66%), followed by France (22%), Greece (8%), and Spain (4%) [7]. *D. repens* spread faster than *D. immitis* from the endemic areas of southern Europe to northern Europe [8].

Risk factors and predispositions are not clear and well defined. The number of dogs in a given area, the prevalence of infection, the number of infected mosquitoes, and human exposure can contribute to the spread of the disease in certain geographical areas. It is believed that the risk of human pulmonary dirofilariasis is greater in periods of natural disasters, most likely due to the occurrence of floods, more mosquitoes, and an increase in the number of stray dogs [1]. In Serbia, during a period of 10 years, dog seroprevalence went from 7% to 26.9% (2004–2014) [9].

The anatomical localization as well as the clinical presentation of this parasite varies. Ophthalmic presentation accounts for 40% of reported cases. Nodular localization is found on the head and neck in 18.9–25.3%, in the extremities in 14.8–22.1%, in the torso in 11.4–11.8%, in male genitalia in 2.9–4.1%, in female breasts in 2.5–2.7% of the cases. Cases of unencapsulated forms in peritoneum have also been described in 0.6% of cases [10]. A large majority of patients with dirofilariasis had one painful subcutaneous nodule, without signs of infection. In the case of ocular localization, symptoms are the feeling of burning, itching, and pain in the eye. The majority of patients with pulmonary dirofilariasis are asymptomatic, while 38% have symptoms in the form of cough, fever, and hemoptysis [8].

The diagnosis of the disease is based on the possible visualization of the parasite, as well as the pathohistological verification. VATS has been proven to be the best, both diagnostic and therapeutic, method, because in this way a safe differential diagnosis is performed concerning malignant diseases, tuberculosis, pulmonary thromboembolism, and Wegener's granulomatosis [2].

There are serological tests, but they are rarely available, are not satisfactory, give cross-positive results with other filariases, most commonly with *Toxocar canis* nematode [2]. Bearing in mind all the aforementioned, we may conclude that serological tests should be used exclusively as

supplemental diagnostic procedures. Eosinophilia is present in less than 20% of cases [2]. The differential diagnosis of pulmonary forms of dirofilariasis between a malignant tumor and benign lesion is a major diagnostic challenge. Usually, lesions are detected in the lower right lung, in the form of subpleural pulmonary nodules, 1–3 cm in size. Pulmonary nodules can be individual, multiple, and bilateral. Their radiological signs are non-specific, which causes differential diagnostic problems that cannot be resolved by diagnostic procedures such as MSCT and nuclear magnetic resonance. However, VATS leads to the ultimate and definitive diagnosis, which puts this diagnostic therapeutic modality first in diagnostics, but also in the treatment of human dirofilariasis. After performing the VATS resection and diagnosis of human dirofilariasis, other therapeutic procedures are not necessary [1].

In conclusion, the treatment is both surgical and conservative. Conservative treatment involves the use of ivermectin or albendazole for four weeks. In many cases, causative therapy is not necessary before the surgery. Tumolskaya et al. [6] suggest that the patient should be given a single dose of ivermectin with three doses of diethylcarbamazine if there is a marked suspicion of dirofilariasis.

The importance of dirofilariasis is increasing with regard to global warming, increased number of pets, and human migrations. The number of the diseased is rising and the geographic distribution is changing, especially in northern Europe, outside the endemic area. In case of subcutaneous nodules or unclear lung changes, dirofilariasis should be considered. VATS is the leading diagnostic surgical procedure concerning dirofilariasis, and a significant therapeutic modality.

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Хумана дирофиларијаза

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САЖЕТАК

Увод Дирофиларијаза је зооноза изазвана нематодом из рода *Dirofilaria*, која је паразит паса и других канида, а човек се инфицира убодом комарца. У Европи се повећава број оболелих ван ендемског подручја. До сада је у свету пријављено више од 2850 случајева хумане дирофиларијазе. У нашој установи су у последњих 20 година потврђена само два случаја хумане дирофиларијазе. Болест се манифестује као кутана, висцеларна и офталмична форма. До појаве првих симптома болести може проћи и више десетина година од настанака инфекције. Дијагноза се може потврдити хистолошки или морфолошки и/или применом молекуларних техника. Лечење је хируршко уклањање паразита и примена антипаразитарне терапије.

Приказ болесника У раду су приказана два случаја инфекције нематодом *Dirofilaria repens*. Прва болесница је више година имала промене на кожи лица миграторног карактера са формирањем нодуларне промене. После инцизије индурације на кожи зигоматичне регије извађен је црв величине 7 *cm*, који је идентификован као *Dirofilaria repens*. Други случај је болесник са израженим кашљем и хемоптизијама, код кога је морфолошким претрагама установљена промена нодуларног изгледа у плућном паренхиму. Патохистолошки налаз екстирпиране промене је показао хронично грануломско запаљење и присуство паразита. Патохистолошки налаз екстирпиране нодуларне промене у целости на месту извађеног паразита је показао да се ради о грануломској инфламацији. Лечење оба болесника је завршено успешно.

Закључак У случају појаве поткожних нодула или нејасних промена на плућима треба мислити на дирофиларијазу. Видеоасистирана торакоскопска хирургија је хируршка процедура која заузима водеће место у процесу дијагностике дирофиларијазе, али и значајан терапијски модалитет.

Кључне речи: дирофиларијаза; паразитоза; видеоасистирана торакоскопска хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Rare complication of primary percutaneous coronary intervention – perforation of the axillary artery

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SUMMARY

Introduction Several arteries can be used as the approach for coronarography or primary percutaneous coronary intervention (pPCI). In patients with acute ST-elevation myocardial infarction (STEMI), when performing pPCI according to the current recommendations, approach artery should be the radial artery. Complications of the transradial approach, such as spasm, asymptomatic occlusion, perforation, nerve damage, arteriovenous fistula, compartment syndrome, and radial artery pseudoaneurysm are described. However, only a few cases describe rare complications of transradial approach such as the perforation of the axillary artery.

Case outline The patient was admitted due to the STEMI. Urgent coronarography found 90% stenosis of the proximal segment of the left anterior descendent branch of the left coronary artery (LAD). During the pPCI, a metal stent was implanted in the proximal segment of the LAD. One hour after the intervention, a hematoma in the right arm was registered with the hemodynamic collapse. Angiography of the left axillary artery showed an extravasation of the contrast. A graft stent was implanted in the area of extravasation. After the intervention, regression of the hematoma was registered. Ten years after the primary intervention, CT coronarography and angiography were performed. The stent in the LAD, as well as in the axillary artery, was without any stenosis.

Conclusion Advanced life expectancy, hypertension, atherosclerosis, anatomical variations, and blood vessel tortuosity contribute to the perforation of the axillary artery, a very rare complication of the radial approach. It is usually treated conservatively. In the case of hemodynamic instability, a stent implantation can be considered, as it was in our case.

Keywords: complication; perforation axillary artery; primary percutaneous coronary intervention; graft stent implantation

INTRODUCTION

Several arteries can be used as the approach for coronarography or primary percutaneous coronary intervention (pPCI). Approach arteries on the arm can be axillary, brachial, ulnar, and the radial arteries, while on the leg it is the femoral artery. Today, the radial artery and femoral artery are most commonly used as the vascular approach in the interventional cardiology. Due to the low risk of acute bleeding, vascular complications, short hospital stay, as well as the greater comfort of the patient, radial artery is now a very popular vascular approach. In patients with the acute ST-elevation myocardial infarction (STEMI), when performing pPCI according to the current recommendations of the European Society of Cardiology, approach artery should be the radial artery if the procedure is carried out by an experienced operator (Class I, the level of evidence A) [1]. This recommendation of the European Society of Cardiology is the result of several studies (RIVAL, RIFLE-STEACS, and MATRICS), which showed the advantages of the transradial approach [2, 3, 4]. Complications of the transradial approach, such as spasm, asymptomatic occlusion, perforation, nerve damage, arteriovenous fistula, compartment syndrome, and radial artery pseudoaneurysm are often described in the literature. However, only a few case reports describe the rare potentially fatal complications of the transradial approach such as the perforation of the axillary artery.

CASE REPORT

A 65-year-old woman was admitted as an emergency case to the Clinic of Cardiology of the Institute of Cardiovascular Diseases of Vojvodina because of the STEMI. Dual anti-aggregation therapy (aspirin, clopidogrel) was administered with the analgesic therapy. Urgent coronarography registered a 90% stenosis of the proximal segment of the left anterior descendent branch of the left coronary artery (LAD). Coronarography was performed with the transradial approach. As a diagnostic catheter, Tiger 5F (Terumo Corporation, Tokyo, Japan) was used with the 0.035 inches and 180 cm SIMPLEX J type hydrophilic guidewire (St. Jude Medical, Saint Paul, MN, USA). During the pPCI, one metal stent of 18 × 3.5 mm (Tsunami Gold,

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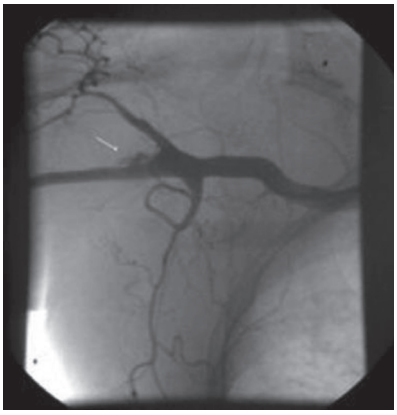

Figure 1. Angiogram of the left axillary artery with extra station of contrast

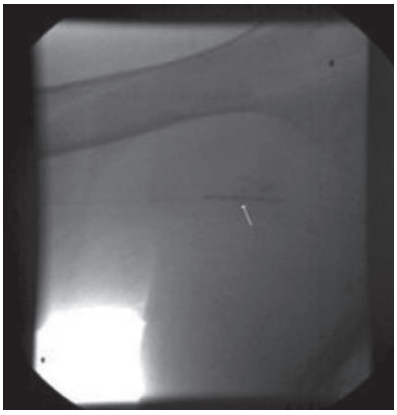

Figure 2. The angiogram shows that the graft stent is positioned distally

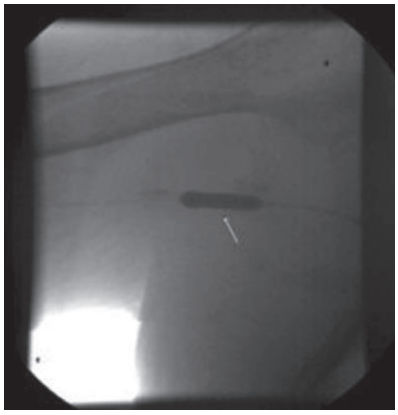

Figure 3. The angiogram shows expansion of the distal part of the graft stent

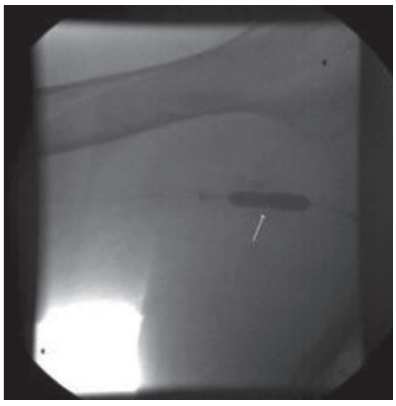

Figure 4. The angiogram shows expansion of the proximal part of the graft stent

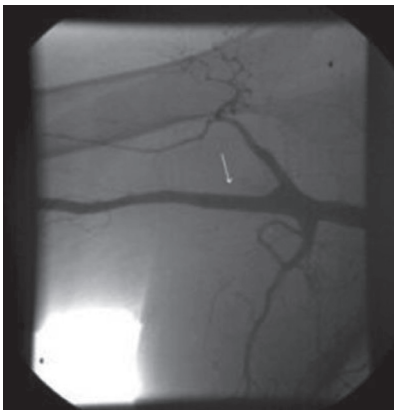

Figure 5. Angiography of the left axillary artery after the implantation of the graft stent was without extravasation of contrast

Terumo Corporation, Tokyo, Japan) was implanted into the proximal segment of the LAD.

Due to the presence of thrombotic masses, inhibitors of the GpIIb/IIIa receptors were administered according to the protocol. About one hour after the intervention, the patient felt pain in the area of the right arm. A growing hematoma of the right arm was registered. The pulses of the radial and cubital artery are filiform. Shortly after that, the hemodynamic collapse developed and the patient became tachycardic and hypotensive. In the laboratory findings, a fall in the parameters of the red blood cells was registered (decrease in hemoglobin by 29%). Crystalloid, colloid, and deplasmated erythrocytes were administered. Inhibitors of the GP IIb/IIIa receptors were stopped. Doppler ultrasonography of the right arm was performed. From the beginning of the branchial artery, enlarged lumen with the suspected flapping intime was registered. The finding of other arm arteries was without any morphological and hemodynamic changes. The interventional cardiologist and cardiovascular surgeon decided to perform the angiography of the right arm arteries.

The diagnostic catheter JR 6F 4.0 (CORDIS, Baar, Switzerland) was used for the angiography, which was guided by the 0.035 inches and 180 cm long guidewire (SIMPLEX J type, St. Jude Medical). At the level of axillary artery, the extravasation of the contrast beyond the lumen of the blood vessel was registered (Figure 1). The catheter AL 1 7F (Launcher, Medtronic, Dublin, Ireland) and the 0.014-inch guidewire (Balance Middle Weight Universal, Abbott Japan Co. Ltd., Tokyo, Japan) were placed. The graft stent of 2.75 × 26 mm (Jostent Coronary Stent Graft, Abbott) was implanted in the area of extravasation of contrast. A stent manually put on a balloon catheter was used. Since there was no balloon of adequate length, a short balloon Ultra-soft SV 5.0 × 20 mm (Boston Scientific, Marlborough, MA, USA) was used. The graft stent was positioned from its distal part (Figure 2). After the first dilatation and the expansion of the distal part of the graft stent (Figure 3), the balloon was withdrawn and the proximal stent expansion was performed (Figure 4). Angiography of the left axillary artery after the implantation of the graft stent was without extravasation of the contrast medium (Figure 5). After the intervention, the regression of the hematoma in the area of the right arm was registered, and the patient was hemodynamically and rhythmically stable, with no subjective discomfort. Control Doppler ultrasonography of the right arm did not register morphological and hemodynamic changes on the upper blood vessels of the right arm. The neurocirculatory finding of the right arm was normal. Ten years after the primary intervention, CT coronarography and angiography were performed. There was stenosis neither in the region of the implanted stents in the LAD (Figure 6) nor in the axillary artery (Figure 7).

DISCUSSION

Transradial approach is associated with a lower incidence of acute bleeding and vascular complications compared with the transfemoral approach. In the RIVAL study, it has been shown that radial approach reduces the incidence of acute bleeding in the acute coronary syndrome, as well as the mortality of STEMI patients [2]. It has also been shown

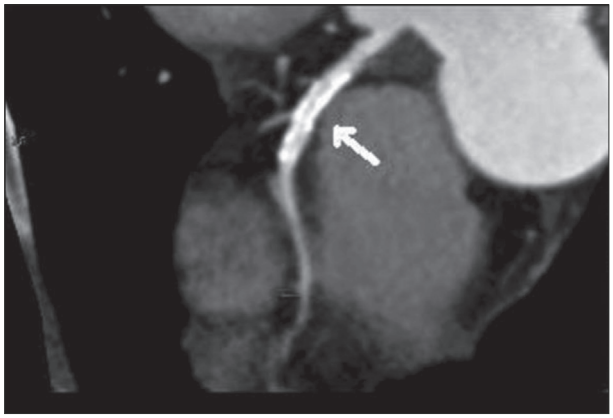

Figure 6. Computerized tomography coronarography performed 10 years after the primary intervention shows no stenosis in the region of the implanted stent in the left coronary artery

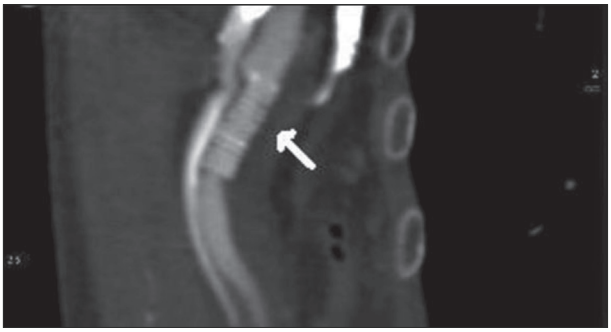

Figure 7. Computerized tomography coronarography performed 10 years after the primary intervention shows no stenosis in the region of the implanted stents in the axillary artery

that the benefit of the radial approach compared with the femoral approach depends on the experience of the operator in the radial approach. The RIFLE-STEACS study has shown that the radial approach reduces the incidence of acute bleeding in the acute coronary syndrome, as well as the mortality of STEMI patients [3]. In the MATRIX study, the patients were randomized for the transradial or transfemoral approach [4]. Radial access is associated with minor bleeding, vascular complications, and the need for transfusion. Patients treated with the transradial approach had a significant reduction of mortality. Also, the disadvantages of radial access, such as the risk of spasm, difficult manipulation with a catheter in the tortoise brachiocephalic tree, movement of catheter during respiration of the patient which can affect the positioning of the stent, longer exposure to X-ray radiation, and the use of catheters of maximal 7F, should not be forgotten.

In the literature, only a few cases of iatrogenic dissection of the axillary artery are described. Advanced life expectancy, hypertension, atherosclerosis, anatomical variations and blood vessel tortuosity can contribute to this rare complication of the radial approach. Forced manipulation guidewire and catheter can also contribute to the perforation of the axillary artery [5]. However, in our case, the perforation most likely occurred during the manipulation of the guidewire. In most cases, perforation occurs when angiography of the mammary artery is performed by a femoral approach. Continuous technological developments including new dedicated guidewires enabling safer and easier interventions [6]. Left radial or brachial approach is the method of choice for angiography of the mammary artery to reduce the risk of subclavian artery damage [7].

The available literature does not describe the algorithm for diagnosis and treatment of subclavian artery dissection. The most common initial diagnosis was set by ultrasonography, and then confirmed by CT angiography. In our case, the patient was hemodynamically unstable and because of that there was no time for CT angiography. It was decided to perform urgent angiography of the right arm arteries.

Perforation of the artery represents a rare complication of the transradial approach. A study that included 10,344 patients who underwent coronarography through the radial artery, in the period from February 2010 to December 2014, found a perforation of the artery in eight patients (0.08%) [8]. Six patients with registered perforation of the radial artery were treated with the mechanical compression. The treatment of the axillary artery perforation is most often conservative – exclusion of anticoagulant therapy and mechanical compression [9]. Emergency surgery was described in only one case of the brachial artery perforation leading to compartment syndrome [8]. In one case, the right internal mammary artery perforation resulting in huge breast hematoma was treated endovascularly with the graft stent implantation [8, 10].

The literature describes only individual cases where subclavian artery dissection is resolved by placing a stent as in our case. Traditionally, the therapeutic option is a prolonged balloon insufflation. In the case of failure with the prolonged balloon insufflation, surgical correction is indicated.

Some cases of iatrogenic dissection of the subclavian artery were successfully treated with a prolonged balloon insufflation [11]. This technique represents an attractive healthcare treatment due to its availability, simplicity, and the lower cost compared to other techniques [11]. Schmitter et al. [12] described stent implantation by an anterograde approach into the spiral dissection area of the subclavian artery. Angiography one day after the intervention registered an extension of the pseudoaneurysm in the middle area of a previously implanted stent, and the stent graft was implanted into this area. Angiography performed two months later showed normal flow through the stent and stent graft. Spies and Fergusson [7] describe the treatment of subclavian artery dissection by placing two overlapping stents using the retrograde approach. Namely, they consider that a retrograde approach is less likely to pass through a false lumen of the blood vessel causing the mechanical lumen extension and dissection extension.

Informed consent statement: Consent was obtained from the patients for publication of this report and any accompanying images.

Conflict of interest: None declared.

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Ретка компликација примарне перкутане коронарне интервенције – перфорација аксиларне артерије

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САЖЕТАК

Увод Већи број артерија се може користити за приступна места за коронарографију, односно примарну перкутану коронарну интервенцију. Код болесника са акутним инфарктом миокарда са *ST* елевацијом (*STEMI*) приликом извођења примарне перкутане коронарне интервенције, према актуелним препорукама, приступна артерија би требало да буде радијална артерија. Описују се компликације трансрадијалног приступа, као што су спазам, асимптоматска оклузија, перфорација, оштећење нерва, артериовенска фистула, компартмент-синдром и формирање псеудоанеуризме радијалне артерије. Међутим, само као појединачни случајеви се описују ретке компликације трансрадијалног приступа, као што је перфорација аксиларне артерије.

Приказ болесника Болесница је примљена због *STEMI*. Ургентном коронарографијом је регистровано 90% сужење проксималног дела предње силазне гране леве коронарне артерије. У истом акту је урађена примарна перкутана коронарна интервенција са имплантацијом металног стента у проксимални део леве коронарне артерије. После интервенције долази до развоја хематома десне надлактице и хемодинамског колапса. Индикована је ангиографија артерија десне руке, којом се у нивоу исходишта аксиларне артерије региструје изливање контраста. Имплантиран је стент-графт у подручје изливања контраста. После интервенције региструје се регресија хематома. Десет година после примоинтервенције урађене су *CT* коронарографија и ангиографија, којима се не региструју сужења у пределу имплантираног стента у левој коронарној артерији, као и у аксиларној артерији.

Закључак Узнапредовало животно доба, хипертензија, атеросклероза, анатомске варијације и тортуозитет крвног суда доприносе перфорацији аксиларне артерије, врло реткој компликацији радијалног приступа. Најчешће се третира конзервативно, али у случају хемодинамске нестабилности болесника и пласирање стента долази у обзир, као у нашем случају.

Кључне речи: компликација; перфорација аксиларне артерије; примарна перкутана коронарна интервенција; имплантација стент-графта

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

The rare manifestation of pulmonary artery agenesis

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SUMMARY
Introduction Unilateral absence of pulmonary artery is a rare vascular malformation. Because of this anomaly, the lungs are supplied by the system of collateral arteries.
Case outline We present a case of the right pulmonary artery agenesis in a female patient. She was admitted to the hospital because of hemoptysis. A computed tomography scan revealed a congenital malformation – the right lung was smaller in size, the right principal pulmonary artery had not been developed along with aberrant tortuous blood vessels.
Conclusion Symptomatic therapy was applied in the case of our patient. There was no need for any surgical treatment. However, in case of massive hemoptysis embolisation or lobectomy/ pneumonectomy will probably be applied.
Keywords: pulmonary arteries; hemoptysis; computed tomography; angiogenesis

INTRODUCTION

Unilateral absence of the pulmonary artery (UAPA) was first described by Frentzel in 1868, and was demonstrated angiographically in 1952 [1]. The most likely theory of UAPA is due to the developmental failure of the sixth left arch, resulting in the absence of the pulmonary artery [2].

Pulmonary artery agenesis can be localized to a single lobe, also can affect an entire lung or in very a rare case both lungs. Because of that, the lungs are supplied by the system of collateral arteries from bronchial, subclavian, intercostal and coronary arteries .The increased pressure in the collateral arteries may lead to pulmonary arteries damage, endothelial tissue damage, and pulmonary arterial hypertension. These submucosal collaterals hypertrophy with time and may rupture causing hemoptysis, which is the most common symptom [3–7].

Congenital UAPA is an extremely rare anomaly (includes approximately 0.39% of all congenital heart diseases), with a prevalence of 1 in 200,000 young adults [3]. This malformation occurs less often when compared to the total anomalous pulmonary venous connection (TAPVC) that is 0.7–1.5% of all congenital heart malformations [8]. Anatomical characteristic of TAPVC is an abnormal connection of pulmonary veins with systemic venous circulation, but so far, no association has been described with UAPA [8].

UAPA is usually associated with other cardiovascular abnormalities (tetralogy of Fallot, ventricular septal defect, coarctation of the aorta, subvalvular aortic stenosis, scimitar syndrome).

It is, in most cases, diagnosed in childhood. The average age of UAPA patients is 14.

However, because of the atypical symptoms, some patients can be diagnosed in adulthood with the symptoms: hemoptysis, dyspnea, reduced exercise tolerance, recurrent bronchopneumonia. In some patients, chronic infection can lead to bronchiectasis [9–12].

Treatment options include revascularization surgery, pulmonary vasodilator therapy (for pulmonary hypertension), pneumonectomy, or lobectomy, embolisation of collateral hemorrhage [11–16].

CASE REPORT

A 28-year-old female, a non-smoker, was admitted to the Clinic for pulmonology, at the Clinical Center of Serbia for evaluation of hemoptysis, which had first occurred four years ago. Hemoptysis, mostly during exertion, repeated again at the end of 2013 and early 2014, but the patient did not visit a doctor. In the meantime, until admission to the hospital, the patient led a normal life; she had a healthy baby by a normal delivery.

Physical examination was unremarkable. Her vital signs were normal, with oxygen saturation of 97% on room air. Her lungs were clear to auscultation, only over the basal part of the right lung the sound was weakened, without wheezes or rales. Her cardiovascular exam revealed normal heart rate and rhythm. Laboratory data showed normal complete blood count and chemistry.

On posteroanterior chest X-ray, the mediastinal shadow and heart were shifted to the right,

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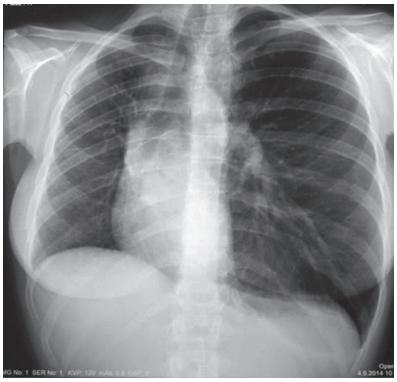

Figure 1. The chest X-ray radiography reveals the mediastinal shadow, the heart that had shifted to the right, and the volume of the right lung is smaller with hyperinflated left lung

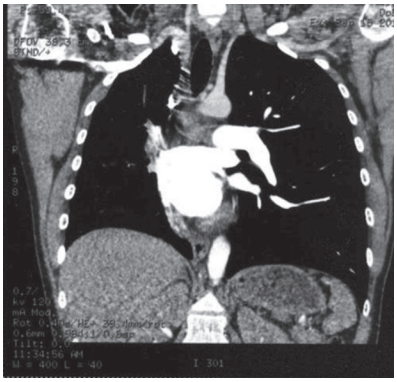

Figure 2. The computed tomography scan shows the pulmonary trunk after leaving the right atrium continues to the left principal pulmonary artery, while the right had not been developed (regular computed tomography scan)

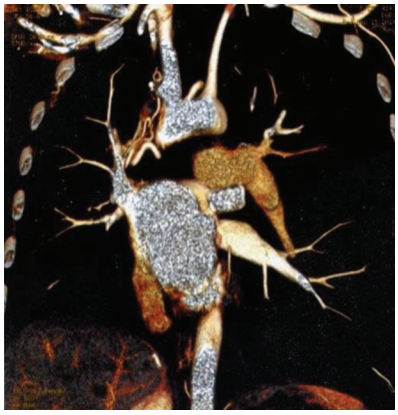

Figure 3. The color 3D computed tomography scan showes the pulmonary trunk after leaving the right atrium continues to the left principal pulmonary artery while the right had not been developed

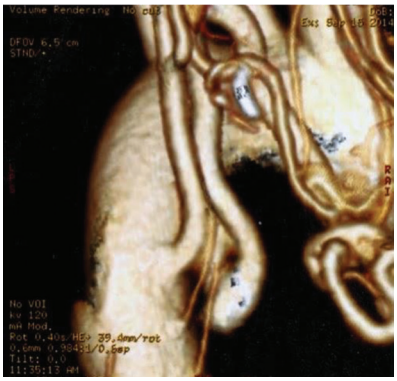

Figure 4. The computed tomography scan shows three main collateral from the descedent aorta

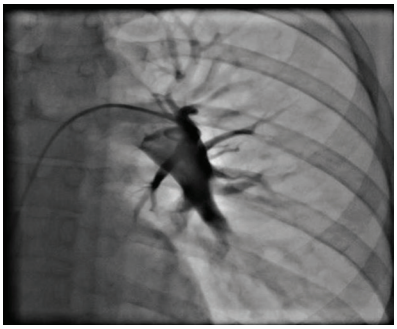

Figure 5. Selective aortography: multiple aortopulmonary collateral arteries

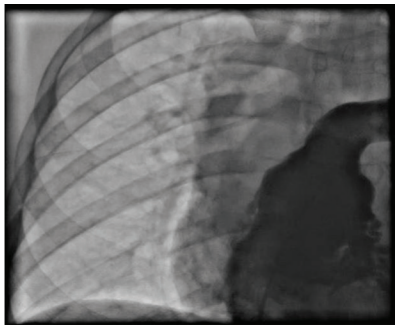

Figure 6. Selective aortography – absence of the right pulmonary artery on aortography

the volume of the right lung was smaller with hyperinflated left lung (Figure1).

The total lung capacity amounted to 106% of predicted, residual volume to 101%, and a diffusion capacity for carbon monoxide to 72% of predicted.

A perfusion scintigram showed the absence of perfusion in the right lung.

Bronchoscopy was normal (no deformity in the bronchial tree, normal arborization of the airways), but incidental finding during bronchoscopy was nasal polyps that tend to bleed.

Echocardiography showed that dimensions of all cardiac chambers were normal. Atrial septum was normal with the suspected ductus arteriosus persistent, and there was absence of the right pulmonary artery. There was no pulmonary hypertension.

Hemodynamic examination (coronary angiography, right ventriculography) revealed tricuspid regurgitation 2+, pulmonary angiography revealed aplasia of the right pulmonary artery while the left pulmonary artery arborization was normal. Aortography revealed ductus arteriosus persistent. There was normal pressure in the right and left heart ventricles.

Another notable point of this case is that the CT scan revealed the congenital malformation – the right lung was smaller in size, developed with all three lobes, the left lung was hyperinflated (Figures 2, 3, and 4). The pulmonary trunk continues to the left principal pulmonary artery after leaving the right atrium, while the right has not been developed. Immediately below the aortic arch are two separating aberrant tortuous blood vessels. Medial blood vessel is dominant and supplies the greater part of the right lung. All four pulmonary veins drain into the left atrium.

We found the existence of multiple aortopulmonary collateral arteries on selective aortography. The three main collaterals come from decedent aorta and supply all three right lung lobes, and there is a secondary collateral from the brachiocephalic trunk which supplies the smaller parts of the upper right lobe. All collaterals were very tortuous, but at no time did it display the right pulmonary artery (Figures 5 and 6).

DISCUSSION

We report a case of a young woman, who was admitted to the hospital because of hemoptysis. During the hospital treatment, we found that the patient had UAPA.

Patients with UAPA can remain asymptomatic for a long period, or may include hemoptysis (in 20% of patients),

dyspnea in physical activity, recurrent respiratory infections, chest pain, or pleural effusion. The most common symptom is hemoptysis, though a massive and life-threatening hemoptysis could also occur. Pulmonary hypertension was diagnosed in 25% of the patients with UAPA and it is a poor prognostic sign [9].

Sometimes hemoptysis can be provoked by factors such as exercise or during pregnancy. Although our patient had one term delivery, she had no complaints.

Our patient had an isolated case of UAPA without any other cardiovascular anomaly. Optimal management requires a multi-disciplinary approach for diagnostic and treatment.

For diagnostics, chest radiography, multiple detector computed tomography scan, magnetic resonance imaging, ventilation-perfusion scintigraphy, and angiography can be used.

On the chest X-ray, there can be a reduction in the volume of hemithorax, an elevation in the hemidiaphragm and mediastinal shift in the affected side [3].

A ventilation-perfusion scintigraphy is rarely performed today and it can show absence of perfusion on the affected lung with normal ventilation [3].

Angiography is the gold standard for establishing a definitive diagnosis and identifying the collaterals in the affected lung [12]. In our case, the blood supply to the affected lung comes from a branch of the artery from the descending aorta, forming collateral circulation for the aorto-pulmonary artery.

Symptomatic treatment consists of medications such as antibiotics, expectorants and bronchodilators, the treatment of pulmonary hypertension and any other treatments for complications. Nowadays in such cases, prophylaxis is very important for respiratory syncytial virus, pneumococcus, and influenza infections [15].

Surgical UAPA treatment methods are revascularization (a systemic-pulmonary shunt involving the hilar arteries), lobectomy, pneumonectomy, and embolization of the developed aorto-pulmonary collateral arteries. In addition, reanastomosis of the peripheral pulmonary arteries, and the pulmonary trunk have been described in literature [11, 16].

We applied symptomatic therapy only according to our council's decision (pulmonologist, thoracic, and cardiovascular surgeons). There was no need for any surgical treatment at that moment because it was not a case of massive hemoptysis.

We advised the patient to visit the otolaryngologist because it is possible that the cause of hemoptysis was nasal polyps that bleed easily when touched. The mucosa of the polyp that was seen by bronchoscopy is very fragile and bleeds easily.

The patient will do follow-ups with a pulmonologist, thoracic, and cardiovascular surgeons. Given the fact that it was an innate birth defect detected at an older age, the symptoms (hemoptysis) are minimal, and mainly during physical exertion and since the patient had had a successful birth, it can be expected that massive hemoptysis is not going to occur. However, we advised the patient that in case of massive hemoptysis, she should immediately report to the hospital in order to decide on further treatment. In case of a massive hemoptysis, surgeons will probably apply embolectomy of the blood vessel that bleeds. As a final option, they might take into account lobectomy or pneumonectomy.

Unilateral absence of pulmonary artery is a very rare vascular malformation and may remain undiagnosed for prolonged periods. Although this malformation occurs in childhood, it manifested itself after 25 years, after our patient had become a mother. A delivery is like any other kind of activity, a risk factor for hemoptysis because the lungs are supplied by the system of collateral arteries. Therefore, it is necessary to think about UAPA in patients with other heart anomalies and if there is a reduced transparency or volume of one lung on a chest radiograph. Optimal management requires a multi-disciplinary approach for diagnostics and treatment. In the case of our patient, only symptomatic therapy was applied. There was no need for any surgical treatment. However, in case of massive hemoptysis, embolisation or surgical treatment of that lung can be applied.

Conflict of interest: None declared.

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Ретка манифестација агенезије плућне артерије

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САЖЕТАК

Увод Једнострано одсуство плућне артерије је ретка васкуларна малформација. Због ове аномалије плућа се васкуларизују системом колатералних артерија.

Приказ болесника Приказујемо редак случај агенезије десне плућне артерије код болеснице, која је иницијално хоспитализована због манифестних хемоптизија. Компјутеризована томографија грудног коша је показала конгениталне малформације, мању димензију десног плућа (десна главна плућна артерија није била развијена) и аберантне крвне судове.

Закључак У случају наше болеснице примењена је симптоматска терапија. Није било потребе ни за каквим хируршким третманом. Међутим, у случају масовних хемоптизија вероватно ће се применити емболизација или лобектомија/пнеумонектомија.

Кључне речи: плућне артерије; хемоптизије; компјутеризована томографија; ангиогенеза

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Sclerosing angiomatoid nodular transformation of the spleen – an uncommon splenic pseudotumorous variant

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SUMMARY

Introduction Sclerosing angiomatoid nodular transformation is a benign splenic pseudotumorous multinodular vascular proliferation. In the past, it was usually reported as splenic hamartoma, multinodular hemangioma or splenic hemangioendothelioma. Since it was defined in 2004, a total of 150 cases have been reported. We will present our experience with a 58-year-old female patient who underwent splenectomy due to the tumorous change in the upper pole of the spleen, histopathologically characterized as sclerosing angiomatoid nodular transformation of the spleen.

Case outline A 58-year-old women presented with abdominal pain, anemia, elevated C-reactive protein and fibrinogen level. Abdominal ultrasound and MDCT scan found a well-circumscribed, homogeneous, low-density tumor in the upper pole of the spleen. As the nature of the tumorous change could not be accurately determined and malignancy could not be excluded, splenectomy was performed. Histological findings showed multiple similar nodular foci, hardly discernible from splenic parenchyma, angiomatoid nodules surrounded and separated by partly collagenized fibroblastic areas admixed with mononuclear inflammatory infiltrate in various proportions. All findings were characterized as the coexistence of sclerosing angiomatoid nodular transformation of the spleen and splenic inflammatory pseudotumor.

Conclusion Splenectomy, laparoscopic or open, is an acceptable therapeutic and at the same time diagnostic method. Considering the important role of the spleen in the immune system, partial splenectomy is also an option, especially in children. However, the coexistence of sclerosing angiomatoid nodular transformation of the spleen and inflammatory pseudotumor indicates a careful treatment decision, given the tendency of inflammatory pseudotumor to relapse, and, rarely, the possibility of malignant transformation.

Keywords: spleen; splenectomy; SANT; inflammatory pseudotumor

INTRODUCTION

Sclerosing angiomatoid nodular transformation (SANT) is a benign splenic pseudotumorous multinodular vascular proliferation. This is a rare clinical entity, which was defined quite recently in a study published by Martel et al. [1] in 2004. Searching bibliographic databases (Pubmed, Scopus), a total of 150 cases have been reported so far. It is more frequent in middle-aged women, but it can also occur in children [2, 3]. SANT rarely manifests clinical symptoms. It is usually found by coincidence as an incidental finding during imaging diagnostics due to some other medical condition. Radiologically, it appears as a peculiar splenic tumor, whose nature cannot be accurately determined, despite the use of modern radiological imaging techniques. The diagnosis may be established only after histopathological and immunohistochemical analyses of the tissue specimen. SANT is composed of angiomatoid nodules immersed in a fibrosclerotic stroma [4]. It can be viewed as more of a pathological diagnosis, since, clinically, its nature still remains unclear. There are no reported cases of relapse after splenectomy.

In this paper, we present our experience with a 58-year-old female patient who underwent splenectomy due to the tumorous change in the upper pole of the spleen, histopathologically characterized as SANT. Written informed consent was obtained from the patient.

CASE REPORT

The patient was admitted to the Clinic for Digestive Surgery within the Clinical Center of Serbia on December 15, 2015 due to chronic dull abdominal pain. A few days earlier, an abdominal ultrasound examination revealed a hypoechogenic tumorous change in the upper pole of the spleen together with cholelithiasis. By examining her medical documentation, we found out that the patient was treated for hypertension with a combination of an angiotensin-converting-enzyme inhibitor and diuretic, non-toxic nodular goiter, and pain in the joints. On admission, she was afebrile with

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normal vitals, and no associated nausea, vomiting, or fever was present. Laboratory examinations, including complete blood count, revealed moderate microcytic anemia [hemoglobin (HGB) 97 g/L, red blood cells (RBC) 3.88×10^{12} /L, mean corpuscular volume (MCV) 79 fL, hematocrit (HCT) 0.3, mean corpuscular hemoglobin (MCH) 24.9 pg, mean corpuscular hemoglobin concentration (MCHC) 315 g/L]. Tumor markers (CA 19-9, CEA, AFP, CA 125, CA 15-3, CA 72-4) were all unremarkable. Evaluation of free thyroid hormones in the serum (FT3, FT4) and thyrotropic hormone TSH confirmed that the patient was euthyreotic. Biochemistry test results showed some features of the chronic inflammatory response through moderately elevated C-reactive protein and fibrinogen level, 44.6 mg/L and 5.6 g/L, respectively. Also, beta-2 microglobulinemia was present (2.91 mg/L).

Multiple detector computed tomography (MDCT) examination of the abdomen and pelvis found a moderate enlargement of the spleen of 152 mm in craniocaudal diameter with a well-circumscribed, homogeneous, low-density tumorous change in the upper pole of the spleen, 30 × 42 mm in size (Figure 1). Para-aortic and interaortocaval lymph nodes were enlarged with a maximal size of 10 mm. Partial wall calcification and multiple small stones in the gallbladder were also seen.

Due to peculiar radiological characteristics, the nature of the splenic mass could not be precisely determined nor could the malignancy be excluded. Therefore, we opted for a splenectomy. During an intraoperative abdominal exploration, we found a tumorous mass in the upper pole of the spleen and gallstones. A splenectomy *in situ* and cholecystectomy was performed. The spleen was sent for histopathological examination. After the surgery, reactive thrombocytosis occurred, but, generally, the postoperative period was uneventful. On the seventh postoperative day, the patient was discharged from the hospital. Vaccines against pneumococci, meningococci, and influenza viruses were prescribed in order to prevent a postsplenectomy infection.

Three months after the surgery, laboratory workup showed improvement of anemia (HGB 107 g/L, RBC 4.38×10^{12} /L, MCV 80.3 fL, HCT 0.35, MCH 24.4 pg). Both C-reactive protein (4.8 mg/L) and fibrinogen (3.4 g/L) were within the normal respective ranges. Four years after the surgery, there has been no evidence of recurrence.

The resected spleen measuring 152 × 110 × 60 mm and weighing 400 g revealed a 20 mm nonencapsulated multinodular mass with a yellow-tan fibrotic central starry scar in the upper pole. Multiple similar nodular foci were found throughout, hardly discernible from splenic parenchyma (Figure 2). Histological findings showed angiomatoid nodules surrounded and separated by partly collagenized fibroblastic areas admixed with mononuclear inflammatory infiltrate in various proportions. In addition, there were areas with myofibroblastic proliferation, hypervascularity, and hemosiderosis. No significant nuclear atypia, mitotic activity, or necrosis was found. Immunohistochemical examination showed a mixture of sinusoidal, capillary, and

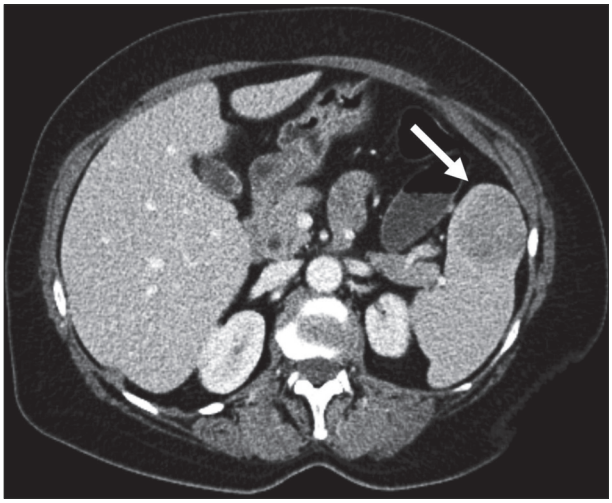

Figure 1. Abdominal computed tomography scan; the arrow points to the change in the upper pole of the spleen

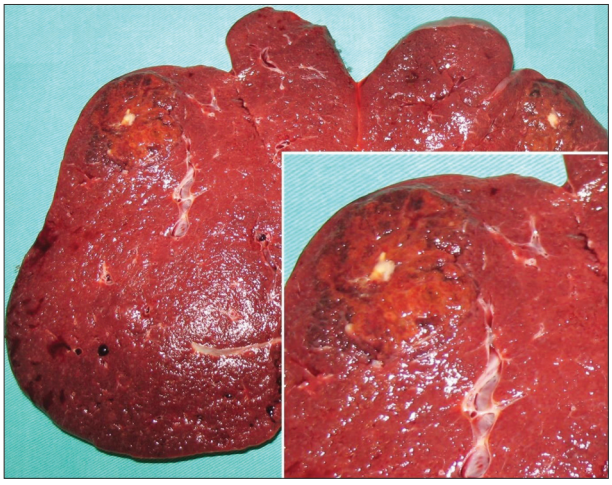

Figure 2. Macroscopic appearance of sclerosing angiomatoid nodular transformation on the cross-section of the spleen

veinlike vessels. Using CD34, CD31, and CD8 antibodies, there were complex endothelial phenotypes resembling splenic sinusoids (CD34-/CD31+/CD8+), capillaries (CD34+/CD31+/CD8-), and small veins (CD34-/CD31+/CD8-). Mesenchymal component revealed nonhomogenous smooth muscle actin immunophenotype and significant immunoreactivity for CD14, CD163, and F-XIIIa. Other antibodies did not express significant reactivity, including desmin, S100 protein, CD117, CD21, CD35, HHV8, fascin, ALK protein, D2-40, and EMA. A portion of mesenchymal proliferation was morphologically and immunohistochemically consistent with inflammatory myofibroblastic tumor (Figure 3).

DISCUSSION

In the past, SANT was usually described as splenic hamartoma, multinodular hemangioma, or splenic hemangioendothelioma. In 2004, in an analysis of 25 cases, a new name was defined by Martel et al. [1] – sclerosing

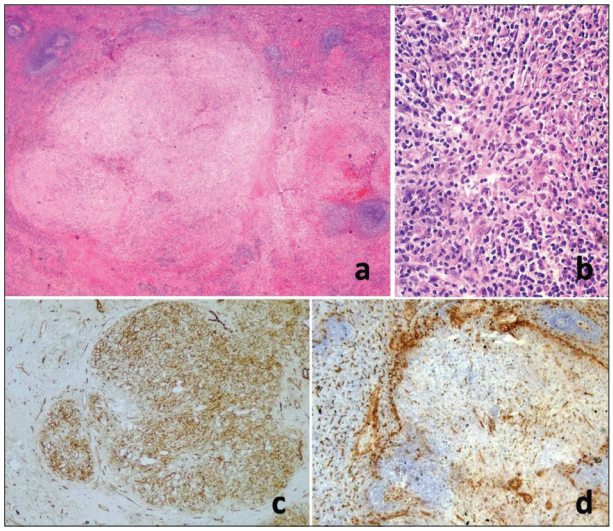

Figure 3. Histological examination of sclerosing angiomatoid nodular transformation clearly depicts nodular transformation of splenic parenchyma (a: H&E, 5×) and in some areas is associated with cellular areas consisting of spindle stromal cells and prominent lymphoplasmacytic infiltrate consistent with inflammatory myofibroblastic tumor (b: H&E, 20×); sclerosing angiomatoid nodular transformation is a result of peculiar reactionary nodular angiomatoid transformation of red pulp with various types of vessels and immunohistochemical expression of vascular antigens such as CD31 (c) and CD34 (d) antigen

angiomatoid nodular transformation of the spleen. In 95% of cases, SANT manifests itself as a solitary splenic lesion. Only five cases of multifocal SANT have been reported to date [5]. In one case report, only the accessory spleen was primarily affected [6].

The etiopathogenesis of SANT has remained unclear to this day. Various authors tried to explain the nature of this lesion. Martel et al. [1] believed that angiomatoid nodules are a transformation of splenic red pulp in response to the interruption of circulation in splenic blood vessels. Diebold et al. [7] constructed the hypothesis that the intrasplenic blood flow disturbance in the red pulp may be a mechanism for the formation of angiomatoid nodules. Weinreb et al. [4] were the first to discern a connection between this disease and Epstein–Barr virus infection. The most recent studies show that SANT results from sclerotic changes accompanied by IgG4-related inflammatory diseases [8]. SANT can also be accompanied by malignant and hematologic diseases, such as polyclonal gammopathy and myelodysplastic syndrome [9].

In our patient, SANT coexisted with splenic inflammatory pseudotumor. Other authors also reported cases of concomitant occurrence of SANT and inflammatory pseudotumors, giving rise to the hypothesis on the close connection between SANT and inflammatory pseudotumor, which show an extremely rare yet possible malignant transformation [10, 11, 12].

Case reports published so far do not offer the possibility to distinguish a prominent clinical characteristic that can be associated with SANT. The most common symptoms that the patients experienced are a sense of abdominal discomfort and occasional dull abdominal pain [13]. Pain in the joints and laboratory analyses of our patient with high levels of C-reactive protein, fibrinogen, and leukocytes may indicate the existence of some type of general inflammatory response. In the study by Diebold et al. [7], three patients out of 16 had laboratory findings indicating inflammation. For one patient, Martel et al. [1] proved a high erythrocyte sedimentation rate, and occasional febrile episodes without a clear cause in two other patients. The existence of moderate iron deficiency anemia and its improvement after splenectomy detected in the case of our patient was also described in the case presented by Budzyński et al. [14].

SANT cannot be radiologically easily distinguished from other vascular lesions, such as the desmoplastic transformation of the splenic red pulp in response to metastatic carcinoma, littoral cell angioma, hemangioendothelioma, lymphangioma, angiosarcoma, hamartoma, and inflammatory pseudotumor. It can be misdiagnosed with splenic abscess [15]. Several cases of splenectomy due to suspected metastatic carcinoma in the spleen have also been described in the literature. However, a histopathologic analysis found SANT, which implies the coexistence of SANT with malignant diseases [8, 16]. Even in the most up-to-date radiological diagnostics such as (^{18}F-labeled fluoro-2-deoxyglucose) positron emission tomography / computed tomography used to follow-up the results of treatment for malignant diseases, radiopharmaceutical accumulation may sometimes falsly detect metastatic disease in the spleen, only to, later, after a splenectomy, establish that it was actually SANT [17]. Macroscopically, on the cross-section of the spleen, the change has a starry aspect. In a large number of reports, the starry shape of SANT has the "spoked wheel" appearance in MDCT and T2-weighted MRI images [18, 19, 20]. However, characteristic and pathognomic radiological findings, which could help unequivocally diagnose SANT, have not been defined yet.

Histopathological findings indicated that SANT can occur simultaneously with splenic inflammatory pseudotumor. Immunohistochemically, one portion of the splenic mass was described as SANT, while the remainder is an inflammatory myofibroblastic tumor. Budzynski et al. [14] reported a case of a patient who had undergone a laparoscopic partial splenectomy due to a splenic tumor, which was later, histopathologically, characterized as SANT. The follow-up proved that this was a satisfactory treatment option. Occasional coexistence of SANT and inflammatory myofibroblastic tumor compromise partial splenectomy as a treatment option. A careful decision should be made, given that an inflammatory pseudotumor can also have recurrence potential, and that rare cases of malignant transformations have been described as well.

Weinreb et al. [4] considered a splenic biopsy to be a reasonable and useful diagnostic method for diagnosing SANT, which is a lesion of vascular nature. Therefore, apart from possible splenic rupture and bleeding, considering coexistence with other inflammatory changes, a splenic biopsy may not be reliable. Angiosarcoma can have very similar radiological findings. Possible peritoneal dissemination is another reason why this biopsy is not advisable. We believe that splenectomy, either classical or

laparoscopic, is at the same time the best diagnostic and therapeutic method.

SANT is a clinical entity with a favorable prognosis. Relapse has not been reported yet. Given that preoperative diagnostics is inconclusive, splenectomy, either laparoscopic or open, is an acceptable therapeutic and at the same time diagnostic method. Considering the important role of the spleen in the immune system, partial splenectomy is also a treatment option, especially in children due to the higher risk of postsplenectomy sepsis [21]. However, the coexistence of SANT of the spleen and inflammatory pseudotumor indicates a careful treatment decision, given the tendency of inflammatory pseudotumor to relapse, and, rarely, the possibility of malignant transformation.

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Склерозирајућа ангиоматозна трансформација слезине – ретка псеудотуморска промена слезине

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САЖЕТАК

Увод Склерозирајућа ангиоматозна нодозна трансформација слезине је бенигна псеудотуморска мултинодуларна пролиферација. Промена је раније најчешће описивана као хамартом, мултинодуларни хемангиом или хемангиоендотелиом слезине. Од 2004. године, када је дефинисан нови назив за ову промену – склерозирајућа ангиоматозна нодозна трансформација слезине, до данас је у литератури описано око 150 случајева. Овај текст, кроз приказ случаја, представља преглед литературе и актуелних сазнања о овом ретком клиничком ентитету.

Приказ болесника Болесница стара 58 година хоспитализована је због хроничних болова у трбуху, анемије умереног степена и умерено повишених вредности *C*-реактивног протеина и фибриногена. Ултразвучним прегледом и мултислајсном компјутеризованом томографијом абдомена виђена је јасно ограничена, хомогена туморска промена у горњем полу слезине. С обзиром на нејасну природу промене и немогућност да се са сигурношћу искључи малигнитет, одлучено је да се уради спленектомија. Хистопатолошка анализа показала је постојање мултиплих нодуларних зона које је веома тешко разликовати од нормалног паренхима слезине, затим ангиоматозне нодулусе који су окружени и раздвојени комбинацијом делимично колагенизованих фибробластних фокуса и мононуклеарног инфламаторног инфилтрата у различитим односима. Такође су виђене зоне миофибробластне пролиферације, хиперваскуларизације и хемосидерозе, што је индикативно за постојање удружених промена, склерозирајуће ангиоматозне трансформације слезине и псеудоинфламаторног тумора.

Закључак Склерозирајућа ангиоматозна нодозна трансформација слезине се радиолошки презентује као тумор слезине нејасне природе. Спленектомија, лапароскопска или отворена, истовремено је метода избора за лечење и начин да се постави дефинитивна дијагноза. Имајући у виду значајне имунолошке функције слезине, парцијална спленектомија се може размотрити, нарочито код деце. Случајеви удруженог постојања склерозирајуће ангиоматозне нодозне трансформације и инфламаторног псеудотумора упућују да одлуку о парцијалној спленектомији треба доносити опрезно, имајући у виду могућност рецидива инфламаторног псеудотумора и његову ретку али могућу малигну трансформацију.

Кључне речи: слезина, спленектомија, склерозирајућа ангиоматозна нодозна трансформација, инфламаторни псеудотумор

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Spontaneous cholecystoduodenal fistula – spectrum of complications

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SUMMARY

Introduction Spontaneous cholecystoduodenal fistula is a rare complication of the gallbladder calculosis. Bowel obstruction is the complication in less than 1% of these patients. The pathognomonic triad (Rigler triad) of pneumobilia, small-bowel distention, and ectopic gallstones is typical for gallstone ileus. In only 1–3% of the patients with bowel obstruction by ectopic gallstone the localization of obstruction is in the duodenum, and it is called Bouveret syndrome. The rarest complication is a floating non-obstructing gallstone trapped in the stomach.

Outline of cases We present three elderly female patients with persistent abdominal pain and known gallbladder calculosis in the patients' histories. Plain radiography of the thorax and abdomen and ultrasound were performed as the first choice and contrast-enhanced computer tomography (CT) was done subsequently. In the first patient, CT and magnetic resonance imaging (MRI) showed signs of pneumobilia, cholecystoduodenal fistula, and the presence of the gallstone in the stomach. The iodine contrast X-ray swallow test revealed a cholecysto-duodenal bulb fistula and floating calculus in the stomach, confirmed by endoscopy. In the second patient with persistent abdominal pain, CT and barium swallow test showed signs of pneumobilia, cholecystoduodenal fistula, and two ectopic gallstones obstructing duodenum – Bouveret syndrome. The third case showed signs of the Rigler triad – typical signs of gallstone ileus.

Conclusion Spontaneous cholecystoduodenal fistula is a rare condition with possible complications such as Bouveret syndrome, gallstone ileus and floating, non-obstructive gallstones in the stomach, as the rarest possible complication. CT, MRI with magnetic resonance cholangiopancreatography, as well as the contrast X-ray swallow test can be very helpful in the detection of the bilio-enteric fistula and ectopic gallstones.

Keywords: cholecystoduodenal fistula; Bouveret syndrome; Rigler triad; complications; ectopic gallstone.

INTRODUCTION

Spontaneous cholecystoduodenal fistula is a rare complication of the gallbladder calculosis, which can cause asymptomatic migrating gallstones within the bowel. Also, in less than 1% of the patients, a bowel obstruction can occur. This entity is more common in the elderly and is manifested with a number of nonspecific signs and symptoms. The pathognomonic triad (Rigler triad) of pneumobilia, small-bowel distention, and ectopic gallstones on conventional abdominal radiographs is seen in 30–35% of all cases with gallstone ileus[1–5]. Duodenal obstruction caused by an ectopic gallstone happens in only 1–3% of the patients and is known as the Bouveret syndrome [1, 6, 7].The rarest complication is a floating non-obstructing gallstone trapped in the stomach, which can also lead to gastric outlet, persistent emesis, and esophageal rupture (Boerhaave syndrome), as reported in the study by Modi et al. [8].

CASE REPORTS

Patient 1

A 77-year-old female with persistent abdominal pain and continuous subfebrile condition was admitted to the Clinic for infectious diseases. Gallbladder calculosis was reported in the patient's history. Blood analyses showed normal leucocyte number and liver function. Abdominal contrast-enhanced computer tomography (CT) was done the following day. CT scan of the thorax and abdomen showed pneumobilia, highly suspected cholecystoduodenal fistula (Figure 1a – arrow) and a 10-mm hypodense ovoid lesion in the stomach with hyperdense rim – calculus (Figure 2a). Magnetic resonance imaging (MRI) examination with magnetic resonance cholangiopancreatography (MRCP) confirmed a cholecystoduodenal fistula (Figures 3a and 3b) and a 12-mmd calculus in the stomach (Figure 3c). Subsequently, an upper gastrointestinal

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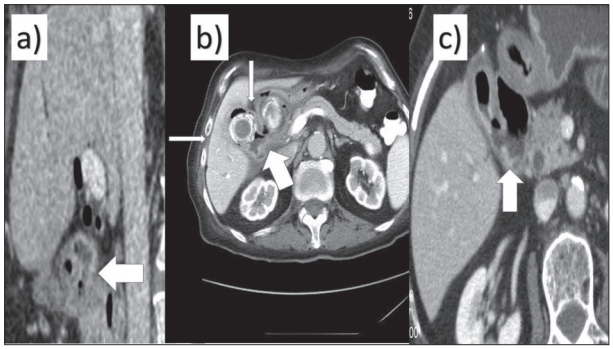

Figure 1. a) Cholecystoduodenal fistula in patient 1 – arrow; b) a 4-cm calculus in the duodenal cap and a 3-cm one in a thick-walled gallbladder (thin arrow), as well as a fistulous formation between the duodenum and the gallbladder (thick arrow); c) cholecystoduodenal fistulization – arrow

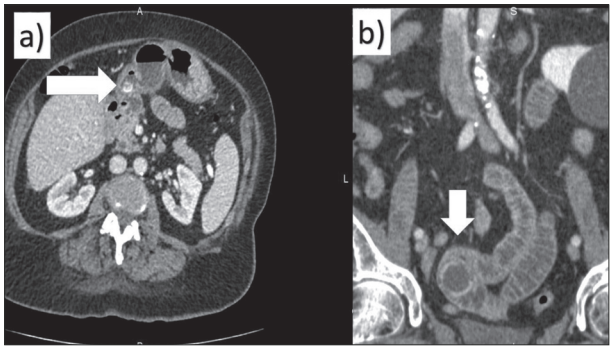

Figure 2. a) Hypodense ovoid lesion in the stomach with hyperdense rim calculus – arrow; b) hyperdense rim, 27–mm gallstone obstructing the ileum – arrow

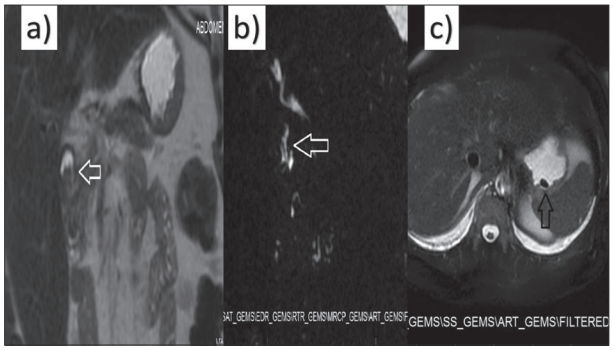

Figure 3. a) Cholecystoduodenal fistula on the T2 sequence on magnetic resonance imaging (MRI) – arrow; b) cholecystoduodenal fistula on magnetic resonance cholangiopancreatography – arrow; c) calculus in the stomach on the T2 sequence on MRI – arrow

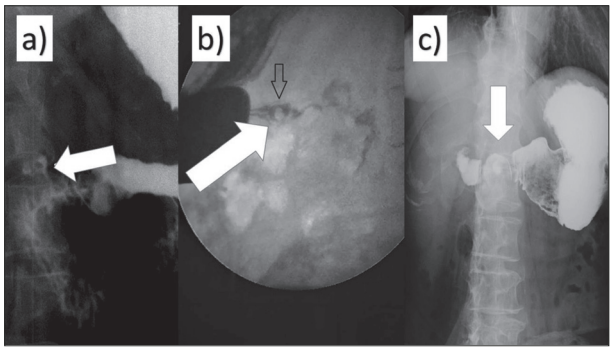

Figure 4. a) Cholecystoduodenal fistula on the X-ray barium test – arrow; b) floating calculus in the stomach on the X-ray barium test – arrow; c) the barium test showed a large filling-defect in the duodenal cap (arrow) with extraluminal passage of contrast from the duodenal bulb

X-ray examination using iodine contrast was performed, which revealed a cholecysto-duodenal bulb fistula (Figure 4a) and a floating calculus in the stomach (Figure 4b). The existence of the gallstone in the stomach was confirmed by endoscopy, but it could not be removed using the basket. Considering the age, clinical condition and the comorbidities, it was decided to only follow-up the patient.

Patient 2

A woman, age 74, was admitted to the hospital with a 10-day history of abdominal pain and recurrent vomiting. Comorbidities included diabetes mellitus, cardiomyopathy, and hypothyroidism. A previous abdominal ultrasound examination showed stones in the gallbladder. Blood analyses showed no signs of leukocytosis and abnormal liver function – therefore, an endoscopic examination was performed.

Patient 3

An 83-year-old female was admitted to our department with repetitive vomiting, abdominal pain, and distention. Gallstones were diagnosed five years earlier; however, it was decided not to perform a surgical procedure due to the poor general and cardiac condition of the patient. Plain radiography of the thorax and abdomen was made. Plain radiography of the abdomen showed small bowel air-fluid levels. The patient was treated only with intravenous fluids and antibiotics. Later, clinical exacerbation was investigated with abdominal CT, which showed signs of pneumobilia, cholecystoduodenal fistulization (Figure 1c – arrow) and contracted irregular gallbladder outline containing air. CT also revealed distended small bowel loops with an ectopic, isodense 27-mm gallstone with hyperdense rim obstructing the ileum (Figure 2b). The gallstone was extracted by means of a longitudinal enterotomy, closed transversally. No attempt was made to remove the gallbladder or to repair the cholecystoduodenal fistula. The patient recovered well.

DISCUSSION

Bilio-enteric fistula is a rare complication which occurs in approximately 1% of all patients with gallstones. Most commonly it happens in older women, which is probably due to the female constitution, as there is regularly elongation and ptosis of hypotonic gallbladder and less visceral abdominal fat tissue around it, so the gallbladder essentially "hangs," which is why it is in direct contact with the duodenal wall. Adding decubitus of the chronically pressured ischemic gallbladder walls (elderly ischemia) with the stones, the cholecystoduodenal fistula is created. Finally, one should be aware of the fact that the presence of sex disparities in some parts of the cardiovascular system anatomy has already been reveled [9].

The most common type is cholecystoduodenal fistula (60%), while cholecystocolic, cholecystogastric, and

choledochoduodenal fistulas are also described [10]. The diagnosis of complications is challenging since clinical signs and symptoms are unclear and unspecific. Patients could suffer from numerous symptoms such as dyspepsia, abdominal pain, malabsorption, melena, and diarrhea. Migrating through the fistula, large gallstones could cause intestinal obstruction, but they could also migrate to any part of the gastrointestinal tract without causing any symptoms. The most commonly obstructed part is the terminal ileum, which leads to the gallstone ileus [2, 4, 10]. Classic abdominal radiographic signs of pneumobilia, mechanical bowel obstruction, and ectopic gallstone were first described by Rigler, but in around one half of the patients there are usually two out of three signs [1, 3, 11]. It rarely happens that patients vomit gallstones passed through fistula to the stomach. There may be a wide range of complications due to an existing fistula such as cholangitis, peritonitis, intestinal obstruction, and hemorrhage due to malignancy [12, 13]. One of the complications of the cholecystoduodenal fistula was presented in our second case – Bouveret syndrome, which implies proximal duodenal or distal stomach obstruction by biliary calculus. It is a rare syndrome that affects elderly women with previous history of biliary calculosis [6, 14, 15].

Oral contrast (iodine or barium) X-ray tests, CT, MRI with MRCP, as well as endoscopic retrograde cholangiopancreatography and hepato-biliary scintigraphy, can be used for diagnosing cholecystoduodenal fistulas [16]. In our cases, the patients had prior knowledge of cholecystolithiasis and were not operated on. CT showed signs of pneumobilia, which indicated the existence of pathological communication between the biliary tree and the gastrointestinal tract [13, 17, 18]. Pneumobilia, gas in the gallbladder and cholecystoduodenal fistula, were also confirmed by the MRI and MRCP examination. MRI with MRCP is useful in detecting isoattenuating gallstones and in patients with the intolerance to oral contrast medium. Negi et al. [19] successfully demonstrated that gallstones, pneumobilia, as well as cholecystoduodenal fistula, can be visualized by this imaging modality.

As demonstrated in our cases, an oral contrast swallow test can be very helpful in confirming the presence of a fistula, an obstructing gallstone in the duodenum as a filling defect, as well as non-obstructing calculi in the stomach. It is a simple and low-cost examination which is highly recommended in cases when a cholecystoduodenal fistula, an ectopic gallstone, and Bouveret syndrome is suspected if the patient can tolerate oral contrast intake [12].

Endoscopy may be performed for both diagnostic and therapeutic purposes as a minimally invasive approach; however, utilization of endoscopy in extracting gallstones using a basket or a net is limited [20]. The primary goal is to eliminate the obstruction (if there is one) by removing the gallstone. Modern management focuses on the less invasive techniques, taking into consideration the patient's age, additional comorbidities, fistula and calculus size, as well as possible complications of more invasive methods such as surgery [21]. In cases of a large calculus, endoscopic removal is often not an option. Furthermore, fragmentation of gallstones with endoscopic graspers may result in fragments migrating to distal parts of the small bowel, leading to new obstruction. Therefore, surgery remains the treatment of choice [22]. A review of 1,001 cases concluded that simple enterolithotomy was both safe and effective in managing patients with gallstone ileus [23]. It is still a matter of debate whether cholecystectomy and repair of the fistula should be performed, due to spontaneous closure of fistulas in some cases [6, 15].

In case of the third patient, after an unsuccessful attempt to remove the floating gastric gallstone endoscopically, the clinicians decided to follow-up the patient having in mind small size of the non-obstructing calculus, poor clinical condition of the patient, and her comorbidities.

In conclusion, one should bear in mind that spontaneous cholecystoduodenal fistula is a rare condition, but that it can have complications such as Bouveret syndrome, gallstone ileus, and floating non-obstructive gallstones in the stomach. CT, MRI with MRCP, as well as the contrast X-ray swallow test can be very helpful in the detection of the bilio-enteric fistula and ectopic gallstones.

Informed consent statement: Consent was obtained from the patients for publication of this report and any accompanying images.

Conflict of interest: None declared.

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Спонтана холецистодуоденална фистула – спектар компликација

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САЖЕТАК

Увод Спонтана холецистодуоденална фистула је ретка компликација калкулозе жучне кесе. Опструкција црева је компликација која се деси у мање од 1% случајева. Риглерова тријада, коју чине пнеумобилија, дистензија танког црева и камен из жучне кесе инклавиран у цревној вијуги, патогномонична је за билијарни илеус. Код 1–3% болесника се опструкција танког црева ектопичним калкулусом из жучне кесе нађе у дуоденуму и то се назива Буверeов синдром. Најређа компликација је неинклавирани камен који се налази у желуцу.

Прикази болесника Приказали смо три старије болеснице са константним абдоминалним болом и познатом калкулозом жучне кесе. Као први дијагностички избор урађен је нативни снимак абдомена и ултразвук, а после њих компјутеризована томографија (КТ). Код прве болеснице КТ и магнетна резонанца су показале знакове пнеумобилије, холецистодуоденалне фистуле и присуство камена у желуцу. Гастродуоденоскопија је показала фистулу између булбуса дуоденума и жучне кесе, као и присуство неинклавираног камена у желуцу, потврђеног ендоскопијом. Код друге болеснице КТ и гастродуоденоскопија баријумом показале су знакове пнеумобилије, холецистодуоденалну фистулу и два ектопична калкулуса који опструишу дуоденум – Буверeов синдром. Код трећег случаја имамо знакове Риглерове тријаде – типични знаци билијарног илеуса.

Закључак Спонтана холецистодуоденална фистула је ретко стање са могућим компликацијама, као што су Буверeов синдром, билијарни илеус или плутајући, неопструишући камен у желуцу, као најређа компликација описана у литератури. КТ, магнетна резонанца и гастродуоденографија могу бити од велике помоћи у откривању билијарно-цревних фистула и камења из жучне кесе на ектопичним локализацијама.

Кључне речи: холецистодуоденална фистула; Буверeов синдром; Риглерова тријада; компликације

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Various faces of the same disease: membranous nephropathy in pregnancy – a case series

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SUMMARY

Introduction Pregnancies in women with membranous nephropathy (MN) are usually complicated by increased proteinuria and superimposed preeclampsia, and this frequently results in poor pregnancy outcomes.

The aim of this paper is to present case series of pregnant women with MN and different fetal and maternal outcomes.

Outline of cases Case 1 presents a 25-year-old woman with MN, who had relapsed nephrotic syndrome in early pregnancy with proteinuria of 4.14 g/day and serum albumin of 30 g/L accompanied by hypertension. Due to a missed abortion, the pregnancy was terminated. Three months later her proteinuria was still increased, measuring 3 g/day.

Case 2 presents a 29-year-old woman with a history of diffuse proliferative glomerulonephritis, who conceived with proteinuria below 0.5 g/day. The proteinuria ranged between 1 and 2 g/day from the 32nd until the 38th gestational week, when she delivered a healthy neonate. After delivery, the woman underwent a kidney biopsy, which revealed MN.

Case 3 presents a 25-year-old woman with MN, whose proteinuria was 1 g/day at the time of conception, but in the 35th gestational week proteinuria of 4.2 g/day was noticed. In the 36th gestational week, increased proteinuria was detected, and a cesarean section was performed with favorable neonatal outcome. After two weeks her proteinuria dropped to 0.6 g/day.

Conclusion Pregnancies in women with MN associated with low-grade proteinuria at the time of conception may have a favorable perinatal outcome. Such pregnancies require multidisciplinary management by both obstetricians and nephrologists, and team decision regarding the best timing of delivery.

Keywords: membranous nephropathy; nephrotic syndrome; pregnancy; preeclampsia; hypertension

INTRODUCTION

The usual complications of pregnancies in women with glomerulonephritis are increased proteinuria, the worsening of hypertension, and development of superimposed preeclampsia, frequently resulting in impaired pregnancy outcomes [1]. Moreover, in some instances such pregnancies may result in progressive deterioration of maternal kidney function. Women with nephrotic syndrome are unlikely to carry the pregnancy until term, although there are reported cases with successful pregnancy outcomes [2]. Nevertheless, modern advances in glomerulonephritis treatment, which increases the frequency of complete and partial remissions, and improved perinatal and neonatal care may allow these women to fulfill their reproductive wishes.

Membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome in adults, and frequently affects women of childbearing age [3]. The course of MN is variable. There are spontaneous remissions, although relapses with deterioration of renal function are common, causing end-stage renal disease in about 40% of idiopathic MN [4].

In a review depicting the outcomes of pregnant women with MN more than 30 years ago, the authors reported that the only predictor of an unfavorable maternal or fetal outcome was the presence of nephrotic proteinuria during the first trimester of pregnancy [2]. These findings were confirmed in later studies, which also concluded that the pregnancy outcomes in women with MN or other chronic glomerulonephritis mainly depend on pre-existing proteinuria before pregnancy [5, 6].

Our work aims to report a case series of different pregnancy outcomes in women with MN.

CASE REPORTS

Case 1

A 25-year-old woman was referred to us for generalized body swelling. She was already diagnosed with MN and regularly followed up by a nephrologist for six years. The disease was initially treated with corticosteroids and cyclophosphamide according to the Ponticelli protocol; cyclophosphamide was replaced by

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mycophenolate mofetil due to recidivant pneumonia. Partial remission was achieved with sustained proteinuria of 2 g/day. Laboratory investigations revealed proteinuria of 4.14 g/day, serum albumins of 30g/L, with normal parameters of kidney function showing serum creatinine (sCr) 69 μmol/L, blood urea nitrogen 4.2 mmol/L, and creatinine clearance (CrCl) 98 mL/min. and abnormal urinalysis (6–8 leucocytes, 1–2 erythrocytes, and 2 granular bodies in a high power field). Her blood pressure was 120/90 mmHg. The patient reported six weeks amenorrhea. Ultrasonography revealed a normal first trimester pregnancy corresponding to her last menstrual period. Due to an overt nephrotic syndrome, pregnancy termination was advised. However, the patient refused this. Therefore, the therapy with angiotensin-converting enzyme (ACE) inhibitors was discontinued, and methyldopa was introduced. Two weeks later, after episodes of high blood pressure of up to 180/130 mmHg, ultrasonography revealed a missed abortion. Except of proteinuria of nephrotic range with hypoalbuminemia, all other laboratory tests, including coagulation status, were quite normal. Dilatation and curettage were performed without complications. Three months later, her proteinuria, although still increased, was in the subnephrotic range (3 g/day); her serum albumin measured 38 g/L. Her kidney function was stable.

Case 2

A 29-year-old patient with a history of diffuse proliferative glomerulonephritis became pregnant after a regular nephrology check-up documenting stable remission of the disease. She was diagnosed with diffuse proliferative glomerulonephritis by kidney biopsy at the age of 19, when she was presented with nephrotic syndrome requiring treatment with corticosteroids. Systemic lupus erythematosus (SLE) was excluded by thorough investigation. Immunologic analysis showed anti-nuclear antibody negativity, and no other laboratory or clinical signs defined by the American Rheumatism Association criteria for SLE diagnosis confirmation were detected. Corticosteroid treatment resulted in decreased proteinuria ranging up to 0.6 g/day. At the age of 26, she gave birth to a healthy neonate by cesarean section (CS) in the 36th week of pregnancy due to the development of nephrotic proteinuria, which subsided to an almost normal range after delivery. Subsequently, she was treated with ACE inhibitors only. Before her second pregnancy, her proteinuria was 0.34 g/day, with serum creatinine of 54 μmol/L and CrCl of 102.9 mL/min. ACE inhibitors were replaced by methyldopa. In the 34th gestational week, she exhibited the rise of proteinuria to the values of about 1 g/day. The patient's blood pressure was normal. The proteinuria ranged 1–2 g/day until the 38th week of gestation. By a planned CS she gave birth to a healthy neonate with birth weight of 2.85 kg and an Apgar score of 10. As her proteinuria did not decrease below 1 g/day for six months, and occasionally even was above 2 g/day, a kidney biopsy was performed again. Histopathology revealed MN with immunofluorescence showing a "full house" pattern. Again, SLE was not confirmed.

Case 3

A 25-year-old patient was followed up during her first pregnancy by a nephrologist due to a history of MN, which had been diagnosed by kidney biopsy 10 years previously. She was successfully treated with corticosteroids and cyclosporine A by a pediatrician nephrologist, and complete remission of nephrotic syndrome was achieved. At 18, when she was presented to an adult nephrologist, her proteinuria level was 0.2 g/day, and normal kidney function and urinalysis were detected. In the further course of the disease, at the age of 23, her proteinuria increased up to 1 g/day without any deterioration of the kidney function and impairment of the blood pressure control. Since her proteinuria remained stable for two years, and her kidney function was normal (serum creatinine was 58 μmol/L and CrCl was 126.7 mL/min.), ACE inhibitors were replaced by methyldopa therapy before being given consent to become pregnant. Until the third trimester, her blood pressure was up to 130/80 mmHg and proteinuria was stable. In the third trimester, continuous rise of proteinuria was observed. Simultaneously, she required higher doses of methyldopa to control her blood pressure that was 150/90 mmHg. The patient was admitted to the obstetric department in the 35th week of gestation, when her proteinuria was 4.2 g/day. The other laboratory tests showed sCr 54 μmol/L, blood urea nitrogen 3.5 mmol/L, CrCl 163 mL/min., total proteins 53 g/L. Ultrasonography revealed intrauterine fetal growth restriction with oligohydramnios and adequate fetal morphology and oxygenation. In the 36th week of gestation, her proteinuria further increased up to 6 g/day and was associated with a decrease in serum proteins to 48 g/L and albumin to 24 g/L. After a dexamethason treatment for fetal lung maturity, a CS was performed in the 36th week of pregnancy and a healthy neonate was born. The birth weight of the neonate was 2.85 kg and the Apgar score was 8. Two weeks later, the patient's proteinuria dropped to 0.6 g/day, and she remained with normal kidney function.

The treatment of all presented patients and reporting of data are in accordance with the ethical standards and the Helsinki Declaration as revised in 2013.

DISCUSSION

Partial remission of nephrotic syndrome, i.e. a fast decrease in proteinuria after abortion, indicates a detrimental role of a pregnancy for sustaining a stable proteinuria level and MN remission. This once again confirms the literature data that the only predictor of a poor maternal and fetal outcome is the presence of nephrotic proteinuria during the first trimester, although the presence of a lower degree of proteinuria is also associated with higher risk pregnancies [2]. Chronic kidney disease, even with normal renal function with low-degree proteinuria, represents a substantial risk for adverse maternal and fetal outcomes, predominantly preeclampsia and iatrogenic preterm delivery. The exact mechanism as to why women

with glomerulonephritis in remission, who have normal renal function and physiologic proteinuria, are much more prone to adverse pregnancy outcomes is not clear. Case 2 and Case 3 have conceived while being in long-time stable remission with normal kidney function and proteinuria of up to 1 g/day. However, in the last trimester, the patient in Case 3 developed a progressive increase of proteinuria accompanied by a drop in the serum albumin and proteins and a rise in blood pressure. These two associated events contributed to intrauterine fetal growth restriction and necessitated preterm delivery.

Only the patient from Case 1 had first trimester pregnancy loss. According to the available studies, the rates of fetal loss in pregnancies complicated by MN range 24–35%, and these occur mostly in the first trimester [1, 7, 8]. A systematic review of six studies showed that the average live birth rate in patients with MN was 86.3%, with only 4% of the fetal losses occurring after the first trimester [8]. However, results regarding adverse maternal or fetal outcomes in pregnant women with MN in the literature are inconsistent. For instance, Packham et al. [2], who analyzed 33 pregnancies, reported a total of 24% of fetal losses, a 43% prematurity rate, and a 33% live birth rate with full-term deliveries.

The severity of glomerulonephritis-induced complications in pregnancy can vary with different pregnancies even in the same woman. The course of the second pregnancy in Case 2 was less complicated than the course of the patient's first pregnancy. Regarding this patient, there is an open question whether she conceived with underlying MN, or it developed during pregnancy. However, the presented course did not urge kidney biopsy during pregnancy, or even therapy with corticosteroids or some immunosuppressive agents.

If nephrotic syndrome is manifested in the first trimester, kidney biopsy can be performed; the treatment of MN can be attempted bearing in mind the possible complications such as, in addition to fetal loss with or without the worsening of maternal kidney function, the appearance of adverse effects associated with immunosuppressive therapy itself [9]. The least toxic immunosuppressive agents used to treat various diseases in pregnancy are azathioprine and cyclosporine. There are several reported cases regarding the treatment of MN in pregnancy, mainly with corticosteroids, which all ended either with preterm delivery or with early elective termination of pregnancy [5, 10, 11, 12]. Cyclosporine used in post-transplant maintenance therapy may be used to treat some forms of glomerulonephritis in pregnancy, including MN [13]. Whether the physician will advise either immunosuppressive therapy or termination of pregnancy in women with high-grade proteinuria depends on several factors. The presence of nephrotic proteinuria in the first trimester poses a high risk for the pregnancy outcome even if immunosuppressive therapy is implemented. If there is hypertension that requires high doses of methyldopa and nifedipine along with nephrotic proteinuria, the chances for a successful pregnancy outcome, even with applied immunosuppressive therapy, is almost negligible. Progression of proteinuria regardless of whether corticosteroids and/or other immunosuppressive medications were applied is an indication for pregnancy termination.

Women with MN are less able to accomplish physiological renal adaptations that are essential for a favorable perinatal outcome, particularly in cases when MN is not in stable remission. Besides the efforts towards decreasing proteinuria in pregnancy, the control of hypertension plays an extremely important role. Superimposed preeclampsia, apart from maternal complications, frequently impairs fetal growth and oxygenation, thus indicating iatrogenic preterm delivery. Despite renal disease, its etiology and duration, the presence of hypertension at the time of conception carries a 10.6 times higher relative risk of fetal loss compared to when blood pressure is well-controlled by therapy, or even when it is normal without therapy [7]. The impact of MN on maternal and fetal outcomes of pregnancy is still unclear given the observed different courses of subsequent pregnancies in patients with MN, or even the spontaneous remission of nephrotic syndrome in pregnant women with MN [14, 15].

In conclusion, preconceptional counseling is essential in women with MN in order to optimize both maternal and fetal outcomes. These patients require multidisciplinary management by both obstetricians and nephrologists, along with careful follow-up, before and after delivery. Women with MN who still have nephrotic proteinuria in spite of conducted immunosuppressive therapy should not be encouraged to become pregnant. In pregnant women, close monitoring is essential to detect the signs of fetal or maternal compromise in a timely manner. One must bear in mind that for a woman with progressive chronic kidney disease who conceives, the index pregnancy might be the last opportunity for childbearing. Timely referral of such patients to tertiary care centers with appropriate neonatal care, due to risks associated with prematurity, is warranted. The appearance of nephrotic proteinuria in pregnant women with previously diagnosed MN poses a significant challenge and requires team decision regarding the best timing of delivery, while still leaving an open question regarding the implementation of immunosuppressive therapy.

Conflict of interest: None declared.

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Различита лица исте болести: мембранозна нефропатија у трудноћи – серија случајева

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САЖЕТАК

Увод Уобичајене компликације трудноће код жена са мембранозном нефропатијом (МН) јесу пораст протеинурије и развој суперпониране прееклампсије, што често резултира неповољним исходом трудноће.

Циљ овог рада је да прикаже серију случајева трудница са МН и различитим феталним и матерналним исходима.

Приказ случајева У првом случају приказана је жена са МН стара 25 година, која је у раној трудноћи имала релапс нефротског синдрома са протеинуријом од 4,14 *g*/дан и серумским албуминима од 30 *g*/*l*, удруженог са хипертензијом. Због изосталог побачаја учињен је прекид трудноће. Три месеца касније протеинурија је и даље била повишена са вредношћу од 3 *g*/дан.

У другом случају приказана је 29-годишња жена са историјом дифузно пролиферативног гломерулонефритиса, чија је протеинурија у време концепције била мања од 0,5 *g*/дан. Протеинурија се одржавала у опсегу од 1 до 2 *g*/дан од 32. до 38. гестацијске недеље, када је рођено здраво новорођенче. После порођаја биопсија бубрега је показала МН.

У трећем случају приказана је 25-годишња жена са МН, чија је протеинурија у време концепције била 1 *g*/дан, али је у 35. гестацијској недељи уочена протеинурија од 4,2 *g*/дан. У 36. недељи трудноће уочен је пораст протеинурије и урађен је царски рез са повољним исходом по новорођенче. После две недеље њена протеинурија се смањила на 0,6 *g*/дан.

Закључак Трудноћа код жена са МН и протеинуријом ниског ранга у време концепције може имати повољан перинатални исход. Ове трудноће захтевају мултидисциплинарни приступ акушера и нефролога, као и тимску одлуку о оптималном тренутку порођаја.

Кључне речи: мембранозна нефропатија; нефротски синдром; трудноћа; прееклампсија; хипертензија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Urrets-Zavalia syndrome following posterior segment surgery – case report and review of literature

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SUMMARY

Introduction Urrets-Zavalia syndrome (UZS) has been defined as a fixed and dilated pupil accompanied by iris atrophy and occasionally secondary glaucoma. The precise cause of the syndrome is uncertain. Most often it has been described following anterior segment surgery.

The objective of this article is to present how to successfully handle patients with UZS after posterior segment surgery. We present all the dilemmas and difficulties we encountered during the diagnostic process.

Case outline This is a case presentation of a patient with UZS following scleral buckle procedure. To our knowledge, this is the first case of UZS following this type of posterior segment surgery. The delay in treatment was mostly due to the lack of knowledge about the linkage of this syndrome with posterior segment surgery. Once the diagnosis was confirmed, parasympathomimetic drops were administered. The patient responded well to the therapy and partial reduction of mydriasis and restoration of pupillary kinetics was observed.

Conclusion Two months after surgery, the treatment of UZS resulted in slight residual anisocoria with signs of iris atrophy. This could indicate reversible mechanism of UZS after posterior segment surgery with iris atrophy as the only permanent consequence.

Keywords: Urrets-Zavalia syndrome; retinal detachment; scleral buckling; pars plana vitrectomy

INTRODUCTION

Urrets-Zavalia syndrome (UZS) has been identified as a fixed and dilated pupil accompanied by iris atrophy and, occasionally, secondary glaucoma. It has been often described following penetrating keratoplasty for keratoconus in patients who have mydriatics in therapy [1]. The precise cause of the syndrome is uncertain. There have been several reported cases of UZS after deep anterior lamellar keratoplasty for keratoconus, Descemet's stripping endothelial keratoplasty for Fuch's endothelial dystrophy, argon laser peripheral iridoplasty, surgical trabeculectomy and phacic anterior chamber intraocular lens implantation [2–5]. The precise cause of the syndrome is uncertain.

We report a case of UZS following scleral buckling surgery, which is, to the best of our knowledge, the first such reported case in the available literature.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

CASE REPORT

A 56-year-old male patient was referred to our clinic because he noticed a "black shadow" in the lower nasal part of the left eye visual field three days prior to the examination. Best corrected visual acuity on the Snellen chart was 0.3 on the left eye and 0.9 on the right one. From teenage years our patient has been myopic: -3.5 Dsph on the right, and -6.5 Dsph on the left eye. Other than high blood pressure, the patient had no health issues.

The examination of the left eye showed retinal tear on the one o'clock with retinal detachment in the upper temporal quadrant. The macula was attached.

The patient was operated on the following day. We performed scleral buckling with equatorial encircling band. On the first postoperative day, the retina was reattached, there was some corneal edema and visual acuity was 3/60. The patient was discharged with standard corticosteroid and antibiotic therapy, but on the next day he returned to our emergency center with extreme pain in the operated eye. His eye lids were swollen, he had corneal edema, slightly wider left eye pupil and inflammatory reaction in the anterior chamber. Intraocular pressure was 30 mmHg and responded well to the administrated local anti-glaucomatous drugs.

On the check-up two days later, intraocular pressure on the patient's left eye was 8 mmHg, there was no corneal edema or inflammatory reaction. We reduced the anti-glaucomatous therapy and 10 days later cancelled it completely because his intraocular pressure was

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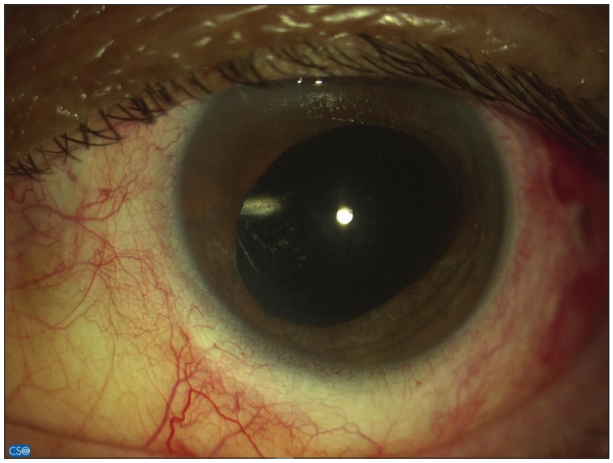

Figure 1. Dilatated irregular pupil and iris subatrophy in the lover part two weeks after the operation

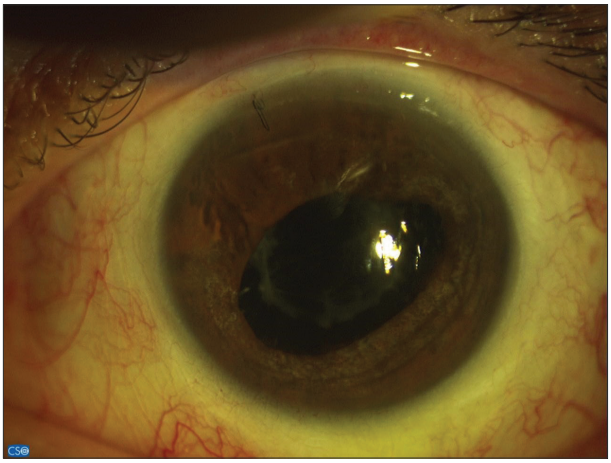

Figure 2. Significantly narrower, still irregular pupil without posterior synechiae and significant opacification of the posterior capsule four weeks after the operation

10 mmHg. The left eye pupil was dilated but we believed it was the effect of the mydriatic eye drops. We were unable to immediately identify the cause of this reaction.

According to the data provided by our patient, his visual acuity improved during the following period. However, in two months he noticed a black shadow in the nasal half of the visual field and he had retinal re-detachment temporally with proliferative vitreoretinopathy. Upon the admission we noticed that the patient's left pupil was dilated, he had posterior synechiae, iris atrophy particularly in the inferior part and incipient anterior subcapsular cataract. The left eye visual acuity was 0.1 on the Snellen chart with a shallow retinal detachment in the macular region. We operated on the patient the next day. Phacoemulsification and pars plana vitrectomy with internal silicon oil tamponade were performed. The patient was discharged on the following day with a normal intraocular pressure and best corrected visual acuity of 0.4 on the Snellen chart. On the check-up two weeks after the surgery, we noticed an even more dilated pupil with wide posterior synechiae and significant iris atrophy (Figure 1).

It was only then that we suspected Urrets-Zavalia syndrome so parasympathomimetic eye drops were introduced three times daily. Three weeks later, the patient had noticed that the left eye pupil was narrower so he reported to the clinic. We registered a significant narrowing of the left eye pupil (Figure 2). The best corrected visual acuity was 0.5 on the Snellen chart and the intraocular pressure was 12 mmHg.

DISCUSSION

To date it has been difficult to explain the UZS etiology following keratoplasty. Past studies examined a number of possible causal factors including strong mydriasis further causing peripheral anterior synechiae and glaucoma, direct iris trauma during surgery, iris ischemia following iris compression between the lens and the cornea during surgery, an abnormal immunological, neurological and iris in keratoconic eyes, intraocular pressure (IOP) rise, preexisting anterior synechiae [2, 6–11]. Furthermore, different studies reported UZS development following complicated diffuse lamellar keratitis with intraoperative microperforation of the Descemet membrane and air bubble in the anterior chamber. It was proposed that the air bubble could cause a pupil block, raised IOP, and secondary iris ischemia with a dilated, fixed pupil [12].

Raised IOP and low ocular rigidity of the eye with keratoconus may cause occlusion of the vessels at the root of the iris within the sclera resulting in iris ischemia, while preserving ciliary body function [10]. This may also be the reason for UZS development in the presented case since scleral buckle may cause transient rise of the IOP, as well as compromise scleral rigidity.

Pathophysiological mechanism of sympathetic spasm with parasympathetic inhibition was suggested since early resolution of UZS with an association of sympatholytic and parasympathomimetic drops has been described in the literature [12].

Since the presented case was the first of its kind, it was diagnosed late so the use of parasympathomimetic drops started two months after primary surgery. We do not know what the permanent consequences of UZS following posterior segment surgery are nor do we know what the deadline for the introduction of topical therapy for avoiding more serious complications is. We started with parasympathomimetic drops two months after primary surgery. The resolution of UZS ensued after three weeks of parasympathomimetic therapy. This could indicate a reversible mechanism of UZS after posterior segment surgery with iris atrophy as the only permanent consequence. The therapeutic effects were determined according to the significant narrowing of the eye pupil and the stabilization of IOP.

What is the best time to start the therapy and how long should it be administered? These questions need answers. Hence, further investigation of this rare disease is warranted.

Conflict of interest: None declared.

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Синдром Уретс-Завалије после хирургије задњег сегмента ока – приказ случаја и преглед литературе

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САЖЕТАК

Увод Синдром Уретс-Завалије (СУЗ) дефинише се као фиксирана и дилатирана пупила праћена атрофијом ириса и повремено са секундарним глаукомом. Тачан узрок овог синдрома је још увек непознат. Најчешће је описан у вези са операцијама на предњем сегменту ока.

Циљ овог рада је да представи како је могуће успешно решити случај СУЗ после хирургије на задњем сегменту ока.

Приказ случаја Ово је приказ случаја болесника са СУЗ који је настао после операције аблације ретине методом склералне копче. Приказани случај је први СУЗ после овог типа операције на задњем сегменту ока. Приказали смо све потешкоће и дилеме које смо имали у постављању ове дијагнозе. Каснији почетак лечења је био због недовољног знања о овом синдрому везаног за операције на задњем сегменту ока. После постављања дијагнозе СУЗ аплицирали смо парасимпатикомиметске капи и добили делимично смањење мидријазе и опоравак пупиларне кинетике.

Закључак Два месеца после операције лечење СУЗ се завршило благом резидуалном анизокоријом са знацима атрофије ириса. Ово указује на реверзибилни механизам СУЗ после операција аблације ретине са атрофијом ириса као трајном последицом.

Кључне речи: синдром Уретс-Завалија; аблација ретине, класична операција аблације ретине; витректомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Primary sinonasal ameloblastoma – a rare cause of unilateral nasal obstruction

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SUMMARY

Introduction Ameloblastoma is a rare, locally invasive benign jaw tumour, originating from odontogenic epithelium, and their presence in the sinonasal tract is usually due to their spread from the gnathic region of the maxilla. Primary sinonasal ameloblastoma is extremely rare, with only a handful of reported cases so far. The objective of this article was to describe a patient with a primary ameloblastoma of the right maxillary sinus and nasal cavity.

Case outline We report a case of a 67-year-old male patient with a year-long history of progressive unilateral nasal obstruction. Clinical and computed tomography examination revealed a mass in the right maxillary sinus and right nasal cavity. After an in-office biopsy under local anesthesia, which suggested the diagnosis of ameloblastoma, the patient underwent complete removal of the mass by a medial partial maxillectomy. Histopathologic analysis confirmed the diagnosis of ameloblastoma.

Conclusion Primary sinonasal ameloblastoma is clinically and radiographically similar to the more common pathology of this particular area and should be included in the differential diagnosis of the unilateral nasal obstruction. The treatment of choice is complete surgical resection. Due to the rarity of the disease, and a small number of cases described so far in the literature, there is still no consensus regarding the optimal surgical technique.

Keywords: ameloblastoma; sinonasal tumour; paranasal sinuses; maxillectomy

INTRODUCTION

Ameloblastomas are rare, locally invasive benign tumours originating from odontogenic epithelium [1]. They account about one percent of all jaw tumours, with mandible more frequently affected than maxilla [2]. Primary sinonasal ameloblastomas without involving the maxillary alveolus are extremely rare. In this article, we describe the case of sinonasal ameloblastoma located in the right maxillary sinus, and in the right nasal cavity, which was initially biopsied in ambulatory setting and subsequently treated by partial medial maxillectomy.

CASE REPORT

A 67-year-old male patient was admitted to our department with a year-long history of a progressive, right-sided nasal obstruction. Relevant medical history included hypertension and glaucoma, for which he was taking regular medication. There was no history of prior sinonasal disease, allergies, or tobacco use. Anterior rhinoscopy revealed a solitary polypoid lesion, originating from the middle meatus (*meatus nasi medius*) and partially involving the common nasal meatus (*meatus nasi communis*) on the right side. The surface of the lesion did not have the typical "glassy" appearance of nasal polyp and was somewhat more firm on palpation. A computed tomography scan of the nasal cavity and paranasal sinuses demonstrated mixed-density mass, completely dimming the right maxillary sinus, with displacement and partial destruction of its medial wall and propagation in the nasal cavity (Figure 1). An in-office biopsy under local anesthesia was performed and initial histopathologic examination suggested an ameloblastoma diagnosis. The patient then underwent the right partial medial maxillectomy through the modified Weber-Ferguson incision (Figure 2). During the operation, the solid and well-defined lesion completely filling the right maxillary sinus was noted, along with its intranasal component, which was observed on initial clinical examination. The lesion was removed in *en-bloc* fashion, together with the medial wall of the maxillary sinus, including the inferior nasal concha. The structures removed resulted in the creation of wide antrostomy, which would further facilitate the postoperative examinations using the rigid Hopkins telescopes. The definitive diagnosis of ameloblastoma was confirmed by histopathology (Figure 3). The postoperative period was uneventful and the patient was discharged four days following surgery. The patient is currently on regular endoscopic follow-ups, which have shown no recurrence of the disease 10 months after surgery.

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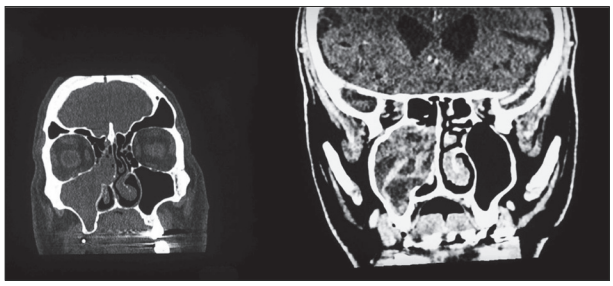

Figure 1. Coronal computed tomography of the nasal cavity and paranasal sinuses (bone and soft tissue windows) demonstrating a mixed density mass in the right maxillary sinus and with an extension to the right nasal cavity

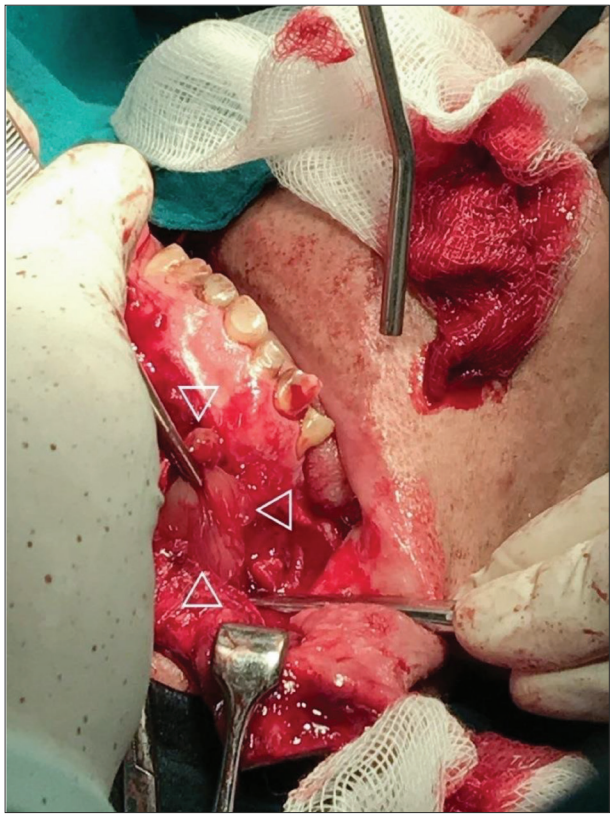

Figure 2. The tumour (triangles) exposed through the modified Weber–Ferguson incision

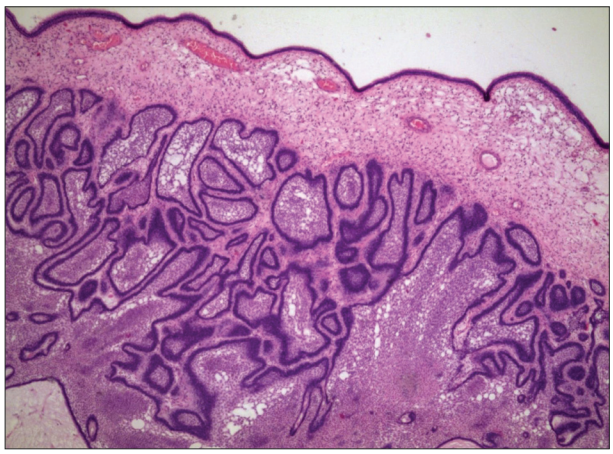

Figure 3. The tumour consisting of loosely arranged stellate cells resembling the stellate reticulum of the tooth germ, surrounded by peripheral rim of palisading cells (hematoxylin-eosin, 25×)

DISCUSSION

Most ameloblastomas in the sinonasal region appear secondary to an extension of a tumour of gnathic origin into this area [3]. However, primary sinonasal ameloblastomas without evident gnathic involvement, have also been described [4, 5]. Unlike their gnathic counterparts, which usually appear between the age of 35 and 45 with no distinct sex predilection, sinonasal ameloblastomas mostly affect male patients in their 60s and 70s [6]. The proximity of the odontogenic apparatus and sinonasal cavity during embryogenesis could potentially result in misplacement of odontogenic cells in the sinonasal epithelium or the abnormal differentiation of the pluripotent basal cells of the sinonasal mucosa. The possibility that it origines from the bony structures of the nasal turbinates has also been proposed [5, 6]. It is unclear whether the chronic inflammation of the sinonasal mucosa may be the triggering event in the pathogenesis of ameloblastoma, or if it is secondary due to tumour presence in the sinonasal tract [7]. Histologically, ameloblastomas are benign neoplasms with locally aggressive behaviour and marked tendency for late recurrence. Cases of malignant alteration and distant metastases, although exceptionally rare, have also been reported [8, 9]. They manifest with nonspecific nasal symptomatology, including progressive nasal obstruction, recurrent epistaxis, facial swelling or sinusitis. On examination, a soft tissue mass in the nasal cavity is usually noted. Clinically and radiologically, sinonasal ameloblastomas are indistinguishable when compared to more common nasal pathology such as polyps, chronic sinusitis, or inverted papilloma. Unilateral nasal involvement and computed tomography signs of bone affection should raise suspicion of a neoplastic process, and definitive diagnosis of ameloblastoma is only possible with a biopsy followed by histopathological analysis. A wide surgical excision is the treatment of choice [10]. The choice of operation is usually dictated by the extent of the disease. A variety of transfacial approaches, such as lateral rhinotomy, sublabial or Weber-Ferguson incisions provide good visual control and enable wide excision. Simple curettage of maxillary sinus is usually associated with recurrence. Wider excisions by means of partial or radical maxillectomy have better outcomes. Recently, endoscopic management of ameloblastomas has been described, with reportedly less perioperative morbidity and better disease control [11].A combined transfacial and endoscopic approach was reported [12]. In the case we described, we have chosen medial maxillectomy through modified Weber–Ferguson incision, based on the involvement of both of nasal cavity and of maxillary sinus, as well as computed tomography scan evidence of bone affection of its medial wall. Currently there is no consensus regarding the choice of surgical technique, because of the small number of reported cases. In cases of infiltrative tumours where complete removal proves difficult or impossible due to its adherence to the surrounding structures, postoperative radiotherapy can be used as an adjuvant treatment [13]. In our case, the tumour was well defined and was completely removed through the selected

approach without difficulties. Radiotherapy could be used as a single therapy in the case of locally advanced disease uncontrollable by surgery alone [14].

Given the benign histologic nature of sinonasal ameloblastoma and its high potential for local recurrence, we would favor the wide surgical excision, which we achieved through transfacial approach. The definitive choice of surgical treatment for sinonasal ameloblastoma is still controversial and requires a larger series of patients. Due to the tumour potential for late recurrence, regular periodic checkups are essential and the exact treatment outcome should only be assessed after a long-term follow-up. In our case, wide communication of the nasal cavity with the remnant of the maxillary sinus was created, thus providing relatively simple follow-up using rigid Hopkins telescopes in outpatient service.

Conflict of interest: None declared.

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Примарни синоназални амелобластом – редак узрок једностране носне опструкције

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САЖЕТАК

Увод Амелобластоми су ретки, локално инвазивни бенигни тумори вилица који потичу од одонтогеног епитела, а њихова појава у пределу носа и синуса је најчешће последица ширења тумора из загрижајног предела горње вилице. Примарни амелобластоми носно-синусне регије су изузетно ретки и до сада је описано свега неколико случајева.
Циљ овог чланка је био да прикажемо случај болесника са примарним амелобластомом десног максиларног синуса и носне шупљине.

Приказ болесника Приказујемо случај мушкарца старог 67 година са прогресивном једностраном носном опструкцијом. Клинички налаз и компјутеризована томографија су указали на експанзивну промену у десном максиларном синусу и у десној носној шупљини. После биопсије у локалној анестезији, која је указала на амелобластом, учињене су парцијална медијална максилектомија и ресекција тумора. Хистопатолошка анализа је потврдила дијагнозу амелобластома.

Закључак Примарни амелобластом носно-синусне регије је клинички и радиолошки сличан чешћој патологији ове регије и требало би га укључити у диференцијалну дијагнозу једностране носне опструкције. Лечење избора је комплетна хируршка ресекција. С обзиром на реткост овог обољења, као и на мали број до сада описаних случајева, тренутно не постоји сагласност о оптималној хируршкој техници.

Кључне речи: амелобластом; носно-синусни тумор; параназалне шупљине; максилектомија

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Dysfunction of the arteriovenous fistula for hemodialysis as a consequence of venous neointimal hyperplasia and treatment strategies

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SUMMARY
One of the main problems related to inadequate planning of vascular access is dysfunction during maturation. Arteriovenous fistula dysfunction is most often a consequence of neointimal hyperplasia. Important causes for initial dysfunction of the fistula include narrow lumens of the arteries and veins used for anastomosis, damage to the vascular endothelium during fistula creation, previous venipuncture, postoperative development of venous collaterals, the impact force of friction on the arteriovenous anastomosis, a genetic predisposition for development of vascular stenosis, neointimal hyperplasia and previously persistent venous neointimal hyperplasia.
Any damage to the endothelium is a stimulus for neointimal hyperplasia. During surgery for creating the fistula, endothelial cells separate on the intima, edema appears, fibrin is deposited, leukocytes and platelets infiltrate. Spotted edema and necrosis of smooth muscle cells appear in the media.
In order to determine an adequate therapeutic strategy, the pathogenesis of intimal hyperplasia has been widely considered from different aspects. It is currently based on preoperative preservation of veins and careful selection of blood vessels, percutaneous transluminal angioplasty or surgical revision. Nevertheless, no current therapeutic strategies provide appropriate recommendations to improve maturation of the arteriovenous fistula. Notwithstanding considerable knowledge about the pathogenesis of venous neointimal hyperplasia, currently no prophylactic treatments would reduce its progression.
Keywords: hemodialysis; arteriovenous fistula; dysfunction; pathogenesis; venous neointimal hyperplasia; therapeutic strategy

FUNCTIONALITY OF ARTERIOVENOUS FISTULAS AND THE MOST SIGNIFICANT COMPLICATIONS

More than 940 patients per million population in Europe are affected by end-stage renal disease and live on chronic renal replacement therapy. Approximately 80% of these patients are treated chronically by hemodialysis. The total number of patients on dialysis in Europe is above 500,000, with an annual increase of 7%. Despite major advances in the treatment of hemodialysis patients, over the past three decades the Achilles' heel of this therapy is creation of the vascular access, which is often followed by significant complications, both during creation and use. Such complications in these patients are among the main causes of morbidity and hospitalization. Therefore, prevention of arteriovenous fistula dysfunction remains an open clinical challenge, with more than 90,000 annual review procedures or reoperations in Europe [1, 2].

Early arteriovenous fistula dysfunction is defined as a fistula that has never been adequately developed for use or which has thrombosed in the first three months of use [3]. Remuzzi et al. [4] have pointed out that the mean annual survival of arteriovenous fistula is 70% (42–90%), and that the primary patency for a period of two years is less than 50%. As the main reason for the dysfunction of arteriovenous fistula is inadequate venous dilation, hemodynamically significant obstruction of the venous conduit or perianastomotic segment, as a consequence of neointimal hyperplasia [5]. Due to the extremely high rates of morbidity in these patients, it is necessary to establish the fundamental pathogenesis of intimal hyperplasia due to possible therapeutic strategies which would prevent this process. All this is associated with a significant financial cost. The cost for the treatment of patients with the end stage kidney disease amounted to $ 24 billion in 2007 in the United States, and for creating vascular access and for resolving complications it amounts to about 1.8 billion dollars a year [6].

Twenty five to 30 years ago, only 10% of new fistulas could not be used, but subsequently this increased to 20–50%. About 28–53% of arteriovenous fistulas for hemodialysis do not achieve proper maturation, despite a waiting period of six months or more. In such cases, further therapeutic procedures involve the use of catheters or arteriovenous grafts, which raises treatment costs and significantly increases the risk of morbidity and mortality [7].

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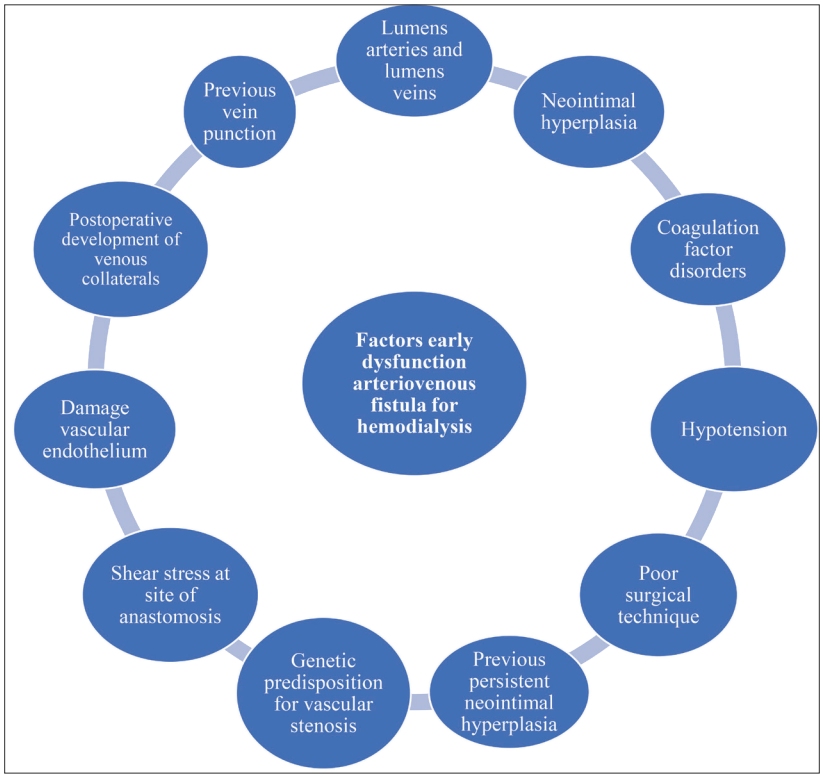

Figure 1. Parameters affecting early dysfunction of arteriovenous fistula for hemodialysis

FACTORS OF ARTERIOVENOUS FISTULA DYSFUNCTION

Numerous studies have given defined reasons important for the initial dysfunction of an arteriovenous fistula (Figure 1). These include small lumens of the arteries and veins used for anastomosis, damage to the vascular endothelium during fistula creation, previous venipuncture, postoperative development of venous collaterals, the impact force of friction on the arteriovenous anastomosis, a genetic predisposition for development of vascular stenosis, neointimal hyperplasia and previously persistent venous neointimal hyperplasia [8]. Some clinical experience suggests that hypotension, coagulation factor disorders and poor surgical technique may affect the initial function of an arteriovenous fistula [9, 10].

A NEW INSIGHT INTO FACTORS ASSOCIATED WITH VENOUS NEOINTIMAL HYPERPLASIA

Patients with chronic kidney disease are characterized by elevated levels of markers of oxidative stress, which implies the occurrence of endothelial dysfunction and vascular morbidity. Hemodynamic forces of friction and damaging therapeutic angioplasty procedures have led to increased synthesis of free radicals and certain bioactive substances, as well as powerful regulators of the key enzyme, matrix metalloproteinase. This degrades the extracellular matrix, facilitating migration of smooth muscle cells in the formation of neointimal hyperplasia [8]. In clinical studies on arteriovenous fistulas with stenosis and thrombosis of the venous conduit, elevated levels of myeloperoxidase were found in the region of intimal hyperplastic stenosis of the fistula [11]. Recent investigations have shown that increased levels of C-reactive protein, high reactivity of interleukin-6 and tumor necrosis factor alpha, and inflammatory cells (macrophages and lymphocytes) may have a possible connection with the extent of neointimal hyperplasia in patients with arteriovenous fistula dysfunction. Also, it has been determined that endothelial function deteriorates in uremic syndrome, a state of permanent inflammation and oxidative stress. In this regard, the concentration of asymmetric dimethylarginine, an important parameter of endothelial dysfunction, was two to six times higher in patients with end stage kidney disease when compared to the general population [8]. All these stenosis-initiating factors result in activation of smooth-muscle cells and fibroblasts in the media, as well as adventitia, with migration and proliferation in the intima. This excess of extracellular matrix causes expansion of intimal hyperplasia, which contributes to the formation of stenosis that almost always leads to vascular access thrombosis [12] (Table 1).

Table 1. Smooth muscle cell activation parameters

Development of venous neointimal hyperplasia
Free radicals
Matrix metalloproteinase
Myeloperoxidase
C-reactive protein
Interleukin-6
Tumor necrosis factor alpha
Inflammatory cells (macrophages, lymphocytes)
Asymmetric dimethylarginine

NEOINTIMAL HYPERPLASIA FORMATION MECHANISM AND PATHOGENESIS

Any damage to the endothelium stimulates neointimal hyperplasia. During surgery to create a fistula, endothelial cells separate, edema occurs, fibrin is deposited and leukocytes and platelets infiltrate at the peeled intima surface. Endothelial cells produce paracrine factors that inhibit the proliferation of smooth muscle cells. Spotted edema and necrosis of smooth muscle cells are seen in the media. After two weeks, the endothelium covers a thickened intima, and the vein suffers arterialization. It becomes rigid, inflexible, with a damaged endothelium, containing less

endothelial relaxing factor and less fibrinolytic activity in the wall, and consequently blood flow is lower. Conditions that contribute to the formation of intimal hyperplasia are repeated puncture of the vein. The most common place to find neointimal hyperplasia is an arteriovenous anastomosis, due to the turbulent blood flow and increased mechanical stress [13].

Current understanding about the pathogenesis of venous neointimal hyperplasia of arteriovenous fistulas, distinguishes the pathophysiological mechanisms as upstream and downstream events. The proximal events characterize initial changes that are responsible for damage to the endothelium, promoting a cascade of different mediators in the distal parts, regulating oxidative stress, endothelial dysfunction and inflammation. These processes are related to:

- surgical trauma during creation of the arteriovenous fistula
- hemodynamically caused mechanical friction forces in the anastomotic region
- bioincompatibility of materials (for arteriovenous grafts)
- damage to the vascular wall (dialysis needle puncture)
- uremic environment, per se, leads to endothelial dysfunction
- repeated angioplasty procedures can cause additional damage to the endothelium [14].

Intimal hyperplasia is a product of the pathogenesis of juxta-anastomotic stenosis of the arteriovenous fistula, which in experimental models, occurs in about three weeks, while in clinical studies it develops between two and six months afterwards [15]. However, some findings indicate that intimal hyperplasia can occur much earlier [16].

Stenosis of a radio-cephalic fistula is most common in the peri-anastomotic area. Stenosis in a brachial-cephalic fistula often occurs in the first part of the juxta-anastomotic region. Lack of dilatation in the distal or proximal vein segment, may have a significant role in the size of the stenosis, particularly in the context of arteriovenous fistula non-maturation. When there is no adequate dilatation a small amount of venous neointimal hyperplasia can cause stenosis [4].

It is interesting that a large number of non-physiological factors may be responsible for initiating and supporting neointimal hyperplasia. It has been established that a sharp anastomosis angle (30°) generates better results and the appearance of less intimal hyperplasia. Not only is frictional force associated with promoting intimal hyperplasia, but also other factors identified in epidemiological studies, such as diabetes mellitus, race, age, peripheral vascular disease and female sex. Cardiovascular disease is also a risk factor for arteriovenous fistula non-maturation [4].

Precursors of myofibroblasts in adventitia of the vein record a sudden mechanical force due to the increase of vascular resistance resulting from arterial flow during the period of rapid adaptation. Other large investigations focused on the effect of surgical trauma and its possible influence on the development of neointimal hyperplasia [17].

HEMODYNAMIC FACTORS AND VASCULAR PATHOLOGY IN ARTERIOVENOUS FISTULAS FUNCTIONING

The vascular endothelium is a dynamic cell medium that is interposed between the wall and the lumen of the blood vessel, and its main functions are maintaining vascular tone and blood flow, preventing vascular inflammation and proliferation of vascular smooth muscle cells, acting fibrinolytic, anti-atherogenic, anti-inflammatory, anticoagulant, and antiplatelet [17, 18]. After creating an arteriovenous fistula, a rapid increase in blood flow occurs as a result of passive vascular distension under the influence of nitric oxide synthase from endothelial cells and consecutive vascular relaxation of smooth-muscle cells with acute vasodilation. These changes simultaneously trigger structural remodeling of blood vessels, causing not only an increase in arterial and venous lumens, but also thickening of the vein wall. The adaptive response to raised blood flow involves increasing blood vessel lumens, in order to reduce the influence of friction to the level before fistula creation [19].

The altered hemodynamic conditions caused by an arteriovenous anastomosis utilize vascular remodeling mechanisms that contribute to the development of endothelial dysfunction. Together with increased oxidative stress, this is an important promoter of inflammation. Endothelial vasodilator activity (nitrous oxide, prostacyclin, bradykinin) is significantly reduced, while synthesis of powerful vasoconstrictors (endothelin, ACE III, free oxygen radicals) increases markedly [18–21].

Vascular calcifications in patients with chronic kidney disease, are a potential cause of vascular pathology. While calcification of the tunica intima classically associates with atherosclerosis, calcification of the tunica media occurs independently of atherosclerotic plaques and may be registered in arteries of any size. Turbulent flow through an arteriovenous fistula stimulates endothelial hyperplasia and encourages the migration and proliferation of vascular smooth muscle cells. Creation of an arteriovenous anastomosis circumvents peripheral resistance and blood flow is significantly increased, which initiates the process of vascular adaptation. On the other hand, non-uniform geometry caused by blood vessel anastomosis quickly changes the direction and strength of the blood stream. In a latero-terminal anastomosis, blood flow is directed from the artery into the vein, with retrograde flow in the distal part of the arterial conduit. In end-to-end anastomosis, the direction of blood flow from the artery into the vein is in the form of the letter "U," which wastes energy through the entire scope of the anastomotic region. Turbulent blood flow in such conditions may, in the vicinity of the anastomosis, create conditions for neointimal hyperplasia [22].

BLOOD FLOW ADAPTATION AFTER ARTERIOVENOUS ANASTOMOSES CREATION

In the first week, after the formation of arteriovenous fistula, comes to an increase in blood flow, an average of

539 ml/min, and almost triple increase in hemodynamically-induced wall shear stress, which results in a progressive enlargement of the lumen of the vein from 2.4 mm preoperatively, to 6.6 mm 12 weeks after the creation of arteriovenous anastomoses [21]. However, since the shear stress is inversely proportional to the size of the lumen, shear stress returns after 12 weeks in the physiological range [4].

CURRENT THERAPEUTIC PROCEDURES TO IMPROVE THE FUNCTIONALITY OF VASCULAR ACCESS

Notwithstanding considerable knowledge about the pathogenesis of venous neointimal hyperplasia, there are currently no prophylactic treatments that would reduce progression of neointimal hyperplasia. So far only a few different and partly effective therapeutic interventions of vascular access stenosis treatments are known, largely because of a lack of understanding of the cellular and molecular mechanisms that lead to the development of neointimal hyperplasia. Treatment of vascular access dysfunction requires a combined surgical, conservative and radiological approach. The first applied therapy was percutaneous transluminal angioplasty, as the best and simplest form of treatment for stenosis. It gives beneficial results for small, calcified arteries with functionalities of 65–96% over a period of one year. Percutaneous access to a thrombosed fistula is increasingly used as an alternative to surgical methods, while a combination of intraoperative surgical thrombectomy and balloon dilatation has recently been introduced. Surgical thrombectomy was initially the main way of treating fistula thrombosis within 24–72 hours of its occurrence and it involves use of the Fogarty catheter [23].

New alternatives in the treatment of vascular access thrombosis is so-called hydrodynamic thrombectomy, and pulse-spray thrombolysis. Thrombolytic therapy by urokinase injection is modified by placing two special catheters with multiple, side openings oriented in opposite directions in the thrombus. Urokinase is applied by pulse spray thrombolysis pharmaco-mechanically in a dose of 250,000–500,000 IU in short pulses, with active withdrawal of the catheter, back and forth [23].

Anticoagulant therapy can be used in patients with recurrent episodes of fistula thrombosis, but always after removal of the anatomical cause of thrombosis. Aspirin (acetylsalicylic acid) may be used as the sole therapy, or in combination with dipyridamole. In therapeutic doses sulfinpyrazone can inhibit the proliferation of endothelial smooth muscle cells. Ticlopidine has been used as an inhibitor of platelet aggregation. There are some indications that statins and Omega-3 fatty acids may have some effect in preventing restenosis but their mechanisms of action are not sufficiently understood and there is no confirmation of the treatment results in wider studies [23, 24].

Certain observations suggest that anticoagulation protocols could be the correct strategy for inhibition of intimal hyperplasia, but finding a balance between acute thrombosis and bleeding is essential. In an experimental (murine) model of arteriovenous fistula the bleeding incidence was 69%, when acetylsalicylic acid was given. Alternative single use of heparin is an efficient method to prevent acute thrombosis, which occurred in a murine model in 3% of cases and bleeding in 7% of cases. However, randomized studies in humans have not confirmed a significant impact of anticoagulant therapy on arteriovenous fistula maturation, although the incidence of early fistula thrombosis decreased in patients treated with clopidogrel. This significantly lowered the incidence of arteriovenous fistulas thrombosis over a period of six weeks, without reducing the time of maturation of four to five months [25].

There is some experience regarding the impact of ACE inhibitors on the incidence of intimal hyperplasia in patients with cardiovascular pathology [26]. Research on hemodialysis patients suggests that inhibiting the enzyme ACE may block proliferation of smooth-muscle cells as an important factor in the prevention of vascular access stenosis [27].

LOCAL THERAPEUTIC TREATMENT OF VASCULAR ACCESS STENOSIS

Vascular accesses are ideal for the clinical application of perivascular therapy because they can be easily applied during surgery and primarily focus on "active" adventitia. When perivascular therapy is applied over the adventitia, lipophilic molecules can quickly diffuse through all layers of the vessel wall. Local therapeutic therapy of vascular access stenosis involves coating with the perivascular drug (paclitaxel), application of gel foam on endothelial cells, gene therapy with vascular endothelial growth factor, recombinant elastase PRT-201 and an Adventa catheter. Since almost all of these therapeutic procedures have been performed in animal models, or under experimental conditions, launching a research initiative (from animals to man) could lead to advances in understanding the mechanisms of neointimal hyperplasia formation and vascular stenosis, which may facilitate the development of new systemic and local therapies [8].

Systematic administration of pharmacological agents requires achievement of high drug levels in blood, in order to ensure a sufficient anti-proliferative effect on the target site. However, large randomized clinical trials are needed to assess the clinical effectiveness of these interventions in preventing thrombosis of the vascular access [15].

INNOVATIVE METHODS OF NEOINTIMAL HYPERPLASIA SUPPRESSION

Modern strategy of vascular access dysfunction treatment involves timely identification of equivalent quantum neointimal hyperplasia. Some studies in animal models with cardiovascular changes examined balloon dilatation together with the application of endovascular radiation and found decreases in both neointimal hyperplasia and restenosis, after angioplasty. Newly formed blood vessels are especially sensitive to the effects of radiation. Recently published results of intra-coronary gamma radiation in

stent restenosis, showed positive findings in 42% of cases. Similar results were obtained after applying beta radiation. Gene therapy could become an effective way of locally treating neointimal hyperplasia. The most important advancement in the treatment of venous neointimal hyperplasia is a stent coated with a polymer comprising an antiproliferative agent. Using sirolimus European multicenter studies in patients with coronary angioplasty have found almost complete absence of stent restenosis. Nevertheless, it is unknown whether the use of stents coated with antiproliferative agents will be an efficient method for regulation of vascular access dysfunction [28].

The protocol and strategy of therapeutic procedures consists of suppressing conditions, which allow proliferation of smooth muscle cells. It was found a significant antiproliferative effect of the methotrexate paclitaxel and sirolimus on development of venous neointimal proliferation [28, 29]. There are initial positive results in the application of infrared radiation for inhibition of intimal hyperplasia [30, 31]. There is an ongoing study that assess the impact of a sustainable strategy of adenovirus, as a mediator in the expression of β-adrenergic receptor kinase C [32].

Likewise, endovascular interventional techniques [8, 33] have proved successful in the treatment of stenotic lesions. There is significant access on the treatment of focal stenosis, which promotes use of percutaneous transluminal angioplasty, also called "cold embolization" [34, 35].

PRT-201 is a recombinant type I of pancreatic elastase, which in animal models, as a fragment of elastin in the blood vessels, results in permanent lumen vasodilation, but today we know that treatment with PRT-201 is not directed to treatment neointimal hyperplasia but to better help inflow dilatation of the artery [36].

The results of certain studies have found that vitamin D exerts a protective effect on the incidence of vascular calcification. Therefore, it could be concluded that percutaneous transluminal angioplasty balloon, coated with antiproliferative receptor for vitamin D, may inhibit the growth of smooth-muscle cells [37].

Nitinol U-clip (Medtronic Minneapolis, MN, USA) is designed to reduce the use of stitches, which would avoid tying knots and facilitate the creation of a circular anastomosis. Nitinol is used in the so-called U-clip, shaped like memory alloys, made of nickel and titanium. It is believed that the inert properties of the material, in combination with reduced surgical trauma, reduce the migration of activated smooth-muscle cells and depositing them in a layer of neointimal hyperplasia. Single suture technique, when creating anastomosis, reduces turbulent flow and intimal hyperplasia, and, in relation to the extension seam, allows dilatation of anastomosis in the radial direction with each systole. However, despite the obvious advantages, it has not been accepted by most vascular surgeons due to additional time required for tying knots and certain technical problems [38].

A recent bioengineering research of human acellular vessels for vascular access for hemodialysis show that it could be "the light at the end of the tunnel." In fact, the study Lawson et al. [39] opens the door to a new way of thinking (on several fronts) in the context of bioengineering blood vessels. As with all new technologies, there must be caution, as long as data is not available through nonrandomized clinical trials that are in progress [39, 40].

CONCLUSION

Arteriovenous fistula dysfunction is defined as a fistula that has never been adequately developed for use or which has thrombosed in the first three months of use.

Any damage to the endothelium stimulates neointimal hyperplasia. Conditions that contribute to the formation of intimal hyperplasia are repeated puncture of the vein, caused by trauma, wall hypoxia and unnatural hemodynamic conditions. The most common place for neointimal hyperplasia is an arteriovenous anastomosis.

Treatment of vascular access dysfunction requires a combined surgical, conservative and radiological approach.

New alternatives in the treatment of vascular access thrombosis is hydrodynamic thrombectomy, and pulse-spray thrombolysis.

Local therapeutic therapy of vascular access stenosis involves gene therapy with vascular endothelial growth factor, recombinant elastase PRT-201 and an Adventa catheter.

Gamma and beta radiation showed positive findings, along with gene therapy could become an effective way of locally treating neointimal hyperplasia. The most important advancement in the treatment of venous neointimal hyperplasia is a stent coated with a polymer comprising an anti-proliferative agent. Positive effects were also identified using Nitinol U-clip, as well as using vitamin D.

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Дисфункција артериовенске фистуле за хемодијализу као последица венске неоинтималне хиперплазије и стратегија лечења

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САЖЕТАК

Један од главних проблема везаних за планирање васкуларног приступа је дисфункција током сазревања. Дисфункција артериовенске фистуле најчешће је последица неоинтималне хиперплазије. Важни разлози иницијалне дисфункције артериовенске фистуле укључују мање лумене артерија и вена које се користе за анастомозу, оштећење васкуларног ендотела током креирања фистуле, претходне венепункције, постоперативни развој венских колатерала, силу трења која настаје након креирања артериовенске анастомозе, генетску предиспозицију за развој васкуларне стенозе, неоинтималну хиперплазију, као и претходно већ постојећу венску неоинтималну хиперплазију.

Свако оштећење ендотела је стимуланс за неоинтималну хиперплазију. Током операције креирања фистуле настају едем ендотелних ћелија, депоновање фибрина и инфилтрација леукоцита и тромбоцита. Појављују се едем и некроза глатких мишићних ћелија у медији.

Да би се одредила адекватна терапеутска стратегија, патогенеза неоинтималне хиперплазије је свеобухватно разматрана, са различитих аспеката. Терапија се тренутно заснива на преоперативном чувању вена и пажљивој селекцији крвних судова, перкутаној транслуминалној ангиопластици или хируршкој ревизији. Ипак, постојеће терапеутске стратегије не пружају одговарајућа решења за побољшање матурације артериовенске фистуле. Упркос значајним сазнањима о патогенези венске неоинтималне хиперплазије, тренутно не постоје профилактички третмани који би смањили његову прогресију.

Кључне речи: хемодијализа; артериовенска фистула; дисфункција; патогенеза; венска неоинтимална хиперплазија; терапеутска стратегија

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CORRIGENDUM

Frequency, severity and type of anemia in children with classical celiac disease: Corrigendum

In the Serbian language version of the Summary of the article that appeared on pages 189–92 of the March–April 2019 issue of the Serbian Archives of Medicine ("Srpski arhiv za celokupno lekarstvo"; Srp Arh Celok Lek. 2019 Mar-Apr;147(3–4):189–92; doi: https://doi.org/10.2298/SARH181203021R) there is an inaccuracy regarding the stated authors of the article, as one of the co-authors (Dejan Nikolić) was, regrettably, omitted; the authors should be in fact listed in the following manner: *Недељко Радловић, Зоран Лековић, Марија Младеновић , Владимир Радловић, Биљана Вулетић, Синиша Дучић, Зоран Голубовић, Дејан Николић, Мехо Махмутовић, Снежана Петровић-Тепић* (Nedeljko Radlović, Zoran Leković, Marija Mladenović, Vladimir Radlović, Biljana Vuletić, Siniša Dučić, Zoran Golubović, Dejan Nikolić, Meho Mahmutović, Snežana Petrović-Tepić). The authors, as well as the publisher, regret this omission.

Учесталост, тежина и тип анемије код деце са класичном целијачном болешћу: *Corrigendum*

У српској језичкој верзији сажетка чланка који је објављен на странама 189–92 у мартовско-априлској свесци из 2019. године часописа „Српски архив за целокупно лекарство" (Srp Arh Celok Lek. 2019 Mar–Apr;147(3–4):189–92; doi: https://doi.org/10.2298/SARH181203021R) постоји нетачност у вези са наведеним ауторима рада, где је један од коаутора (Дејан Николић) нажалост изостављен; исправан списак аутора рада гласи: Недељко Радловић, Зоран Лековић, Марија Младеновић, Владимир Радловић, Биљана Вулетић, Синиша Дучић, Зоран Голубовић, Дејан Николић, Мехо Махмутовић, Снежана Петровић-Тепић. Аутори, као и издавач, искрено жале због наведеног изостављања.

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине - насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5.000 речи, а за претходно и кратко саопштење, приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100-250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings - MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) - if any - should be translated into English as well.

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