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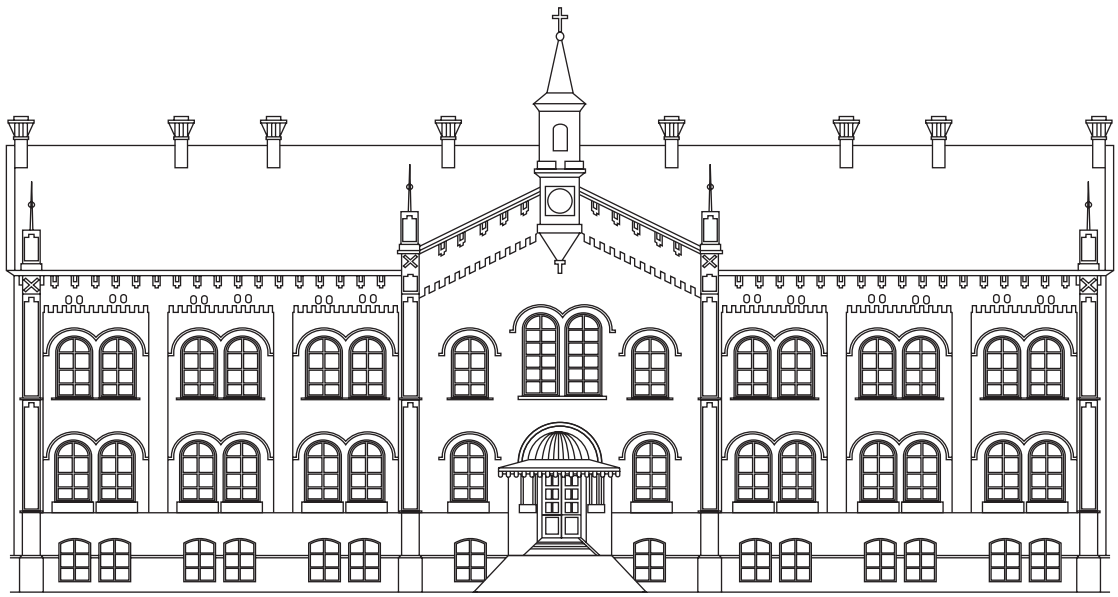

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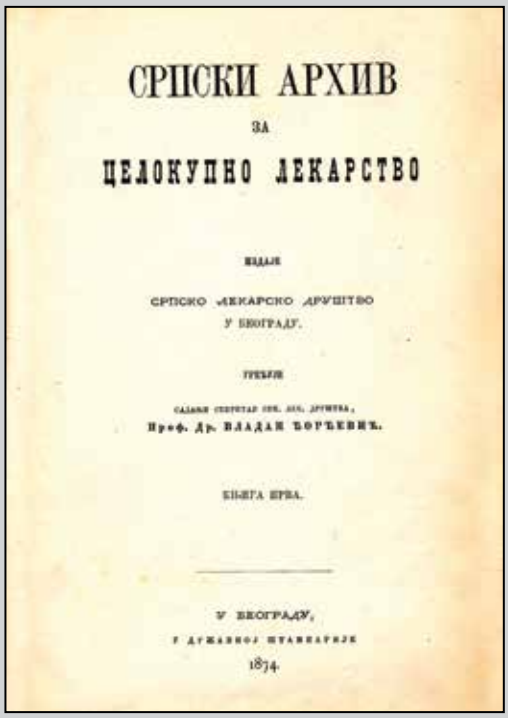

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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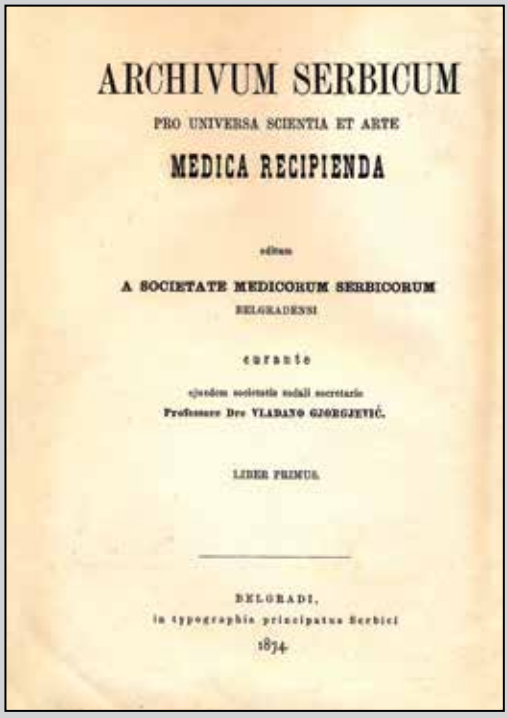

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Selective laser melting and sintering technique of the cobalt-chromium dental alloy

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SUMMARY

Introduction/Objective The objective of this paper is to describe the microstructure and mechanical properties of sintered Co-Cr alloy and to emphasize its advantages and disadvantages with respect to the microstructure and mechanical properties of cast Co-Cr alloy.

Methods Base Co-Cr alloy, EOSint M EOS Co-Cr SP2 (EOS GmbH, Munch, Germany), was used for the purpose of this research as the base material for sintering metal structures of metal-ceramic restorations. Metal sintering was conducted by using EOSint M 280 device of German origin in a stream of neutral gas – argon. After that, the alloy was heated over a period of 20 minutes at the temperature of 800°C. The chemical composition of the alloy was determined by energy dispersive spectroscopy. Microstructure of the tested alloy samples was examined under an optical metallographic and scanning electron microscope. Physical and mechanical properties were measured in a universal testing machine. The samples were prepared according to the standard ISO 527-1:1993.

Results Chemical composition of the sintered Co-Cr alloy, determined by applying energy dispersive spectroscopy, indicated the same qualitative but different quantitative composition compared to cast Co-Cr alloys. The microstructure of the sintered Co-Cr alloy is lamellar in nature, with two dominant phases: ξ-Co and/or ξ-Cr (fcc – *face-centered cubic*) and γ-Co (hcp – *hexagonal close-packed*). Mechanical properties of the Co-Cr alloy obtained by applying selective laser melting technology compared to the cast Co-Cr alloy are superior or approximately the same.

Conclusion Selective laser melting of the Co-Cr alloy is a good example of new technologies based on digitization. Together with other digitized procedures, this technology is an introduction to a new era in dentistry popularly called Dentistry 4.0. The advantages of the selective laser melting technology with respect to the conventional technology of casting Co-Cr alloy metal structures are precise metal structure fitting and eco-friendly technology.

Keywords: selective laser melting; sintering technique; Co-Cr alloy

INTRODUCTION

Dental alloys represent a very dynamic field of dentistry. Changes that occur in this area, in fact, reflect the developments of basic scientific technologies. Mechanical and biological properties of the same alloy are largely dependent on the technological processes of forming the alloy into dental restorations. The process of alloy melting and casting for dental purposes has been known for centuries and melting and casting conditions have been constantly improved – from primitive alloy melting by applying naked flame and open-air casting, to melting by applying induced current in vacuum or neutral gases. Nevertheless, even the perfect casts have certain flaws.

A completely new approach to forming dental restorations appeared with the third, and soon after, with the fourth industrial revolution. The third industrial revolution, also known in the field of dentistry as Digital Dentistry or Dentistry 3.0, introduced numerous new procedures based on digital technologies (3D imaging, intraoral scans, computer-aided design and computer-aided manufacturing – CAD-CAM, cone-beam computer tomography – CBCT, computer-aided implantology). The transition from the third to the fourth industrial revolution, i.e. to Dentistry 4.0, was barely noticeable. Dentistry 4.0 is not a completely new technology. In this case new system solutions were created on the platform (infrastructure) originating from the previous digital revolution. This revolution introduced greater automation in dental laboratory procedures, i.e. diagnostic and therapeutic procedures in dental offices. The selective laser melting (SLM) and compacting (sintering) of metal powder particles is a step forward in the modern dental practice. This technology plunged us into the fourth industrial revolution, i.e. Dentistry 4.0 [1, 2, 3].

The process of making dental restorations by sintering dental alloys basically includes three steps: digital impression, designing virtual restoration, and 3D printing [4–7].

Digital impression, suitable for further computer processing, can be obtained by direct 3D

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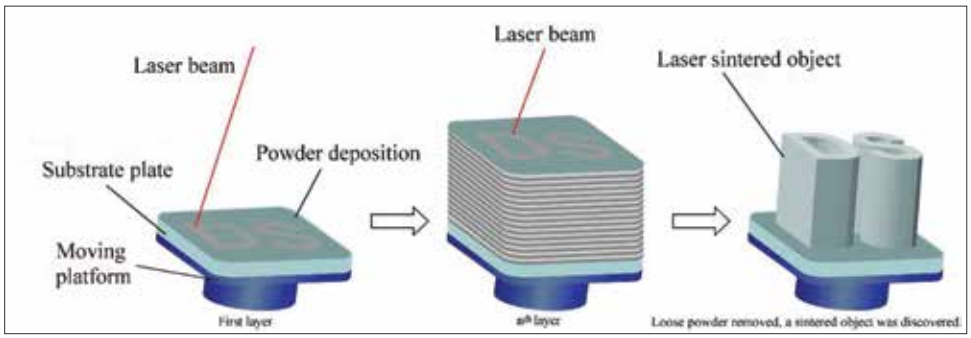

Figure 1. Outline of schematic functioning principles of selective laser melting [7]

digitization in the patient's mouth (intraoral scanners) and indirect 3D digitization of the gypsum model (extraoral scanners) [7, 8]. Nowadays, these scanners, in addition to precision, ensure great comfort for both the doctor and the patient.

Designing virtual restoration, i.e. computerized modeling framework of crown, bridge or removable partial denture, represents the second step in advanced technology of making dental restorations. Virtual restorations are designed by using commercial computer packages, which are computer tools that facilitate and speed up the design process [9]. Virtual restoration files (STL files) are sent directly to the software of the machines designed to make metal frameworks of dental restorations. At this stage, one intermediate step is also possible. If the design control is required in real space, obtained STL files are sent to 3D printers, which print out a model of the future fixed or mobile dental prostheses in polymer or, less often, wax. The detected defects can still be remedied.

The third step is laser melting of the Co-Cr alloy powder and producing the metal framework by sintering. In the process of selective laser sintering, the object is printed by successive addition of thin, horizontal layers. Each layer is printed by applying a thin layer of alloy powder over previously made object, which is then melted with a laser beam in the form of the following layer [10, 11, 12].

Upon cooling, the melted metal powder is bonded horizontally (thus forming a new layer) and vertically (bonding with previously made layer). The form of each layer is determined by a computer, based on a virtual restoration model (obtained STL files). The process of powder melting is governed by cross-sections determined in such manner. The process of sintering only ensures the bond of the laser melted powder (Figure 1) [7].

The objective of this paper is to describe the microstructure and mechanical properties of the sintered Co-Cr alloy and to emphasize its advantages and disadvantages with respect to the microstructure and mechanical properties of the cast Co-Cr alloy.

METHODS

Base Co-Cr alloy, EOSint M EOS Co-Cr SP2 (EOS GmbH, Munch, Germany), was used for the purpose of this research as the base material for sintering metal structures of metal-ceramic restorations. Metal sintering was conducted by using EOSint M 280 device of German origin in a stream of neutral gas – argon. After that, the alloy was thermally treated over a period of 20 minutes at the temperature of 800°C.

The chemical composition of the alloy was determined by energy dispersive spectroscopy (EDS analysis). Microstructure of the tested samples was examined under an optical metallographic microscope (MM) and scanning electron microscope (SEM) in the Materials Testing Laboratory at the Faculty of Mechanical Engineering in Maribor, Slovenia. Physical and mechanical properties were measured in a universal testing machine in the Materials Testing Laboratory at the Faculty of Polymer Technology, Slovenj Gradec, Slovenia. Six samples were prepared according to the ISO standard 527-1:1993.

RESULTS

Chemical composition of the sintered Co-Cr alloy, determined by applying EDS, indicated the same qualitative composition as for cast Co-Cr alloys. However, there were certain differences in the quantitative composition of the alloy, with the values for W, Si, and O being higher (Figure 2, Table 1).

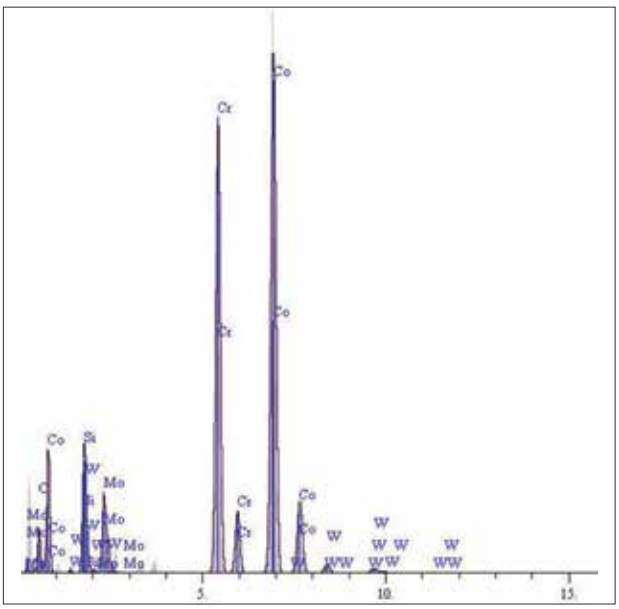

Figure 2. Results of the energy dispersive spectroscopy of EOS Co-Cr SP2 alloy after sintering and thermal treating

Table 1. Numerical values of elements of energy dispersive spectroscopy of EOS Co-Cr SP2 alloy after sintering and thermal treating

Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc.
O	Ka	22.40	0.947	8.878	2.578 wt%
Si	Ka	43.85	1.324	4.954	2.342 wt%
Cr	Ka	404.78	4.024	27.583	26.932 wt%
Co	Ka	511.87	4.525	54.323	58.110 wt%
Mo	La	52.34	1.447	2.813	4.899 wt%
W	Ma	43.78	1.323	1.810	6.038 wt%
			100.000	100.000	wt%

Table 2. Test results for physical and mechanical properties of the selective laser melting (SLM) builds

Samples – SLM		Samples – SLM + thermal treatments	
Tensile strength	800 MPa	Tensile strength	900 MPa
0.2% yield strength	600 MPa	0.2% yield strength	700 MPa
Elongation	10%	Elongation	2%
Modulus of elasticity	170 GPa	Modulus of elasticity	180 GPa

The composition and conditions for compacting particles (sintering) determine the alloy structure. Sintered Co-Cr alloy is examined under MM (Figure 3) and SEM (Figure 4).

Microporosity and porosity, i.e. the presence of dendrites due to contraction, are characteristic for cast Co-Cr alloys. Microscopic examinations of the sintered Co-Cr alloy showed slightly more homogeneous and slightly more porous structure compared to the cast Co-Cr alloy. (Figure 5).

Mechanical properties of the sintered Co-Cr alloy, prior to thermal treating, indicate that the tubes are significantly more brittle compared to the cast Co-Cr alloy. However, after thermal treating, physical and mechanical properties are approximately the same or superior (Figure 6, Table 2). Figure 7 shows the SEM micrograph of the fractured tube surface after mechanical testing.

The roughness of the metal surface is significant, both for the bond between the metal and cement, and for the bond between the metal and ceramics. The roughness of the metal surface concerned ensures better strength of both bonds. SEM micrograph of sintered Co-Cr alloys in this study shows uniform roughness (Figure 8).

DISCUSSION

The results obtained are in accordance with relevant data found in the literature referring to the chemical composition of EOS Co-Cr SP2 alloy determined based on EDS analysis, but also based on X-ray diffractometry analysis (XRD) performed by other authors [13, 14]. Chemical compositions of alloys differ slightly depending on the manufacturer and the surface that the analysis was performed on.

The microstructure of the sintered Co-Cr alloy is lamellar in nature, with two dominant phases: ε-Co and/or ε-Cr (fcc – face-centered cubic) and γ-Co (hcp – hexagonal close-packed). This structure was determined based on XRD analysis [15, 16, 17]. The microstructure of two types of samples is observed: a sintered sample and a sintered and thermally treated sample. The same structure with slightly lower intensity of peaks is determined with the thermally treated sample [17].

Microstructure of the sintered Co-Cr alloy does not indicate intermetallic phases, contrary to the cast Co-Cr alloy. Upon casting, Co-Cr alloys create an intermetallic phase (Cr_7C_3 and $Cr_{23}C_6$) [15]. In theory, the structures obtained by applying SLM technology are not porous. However, this should be taken with some reserve, since the porosity of sintered structures depends on the purity of the input components (alloy powder) and sintering conditions (environment, temperature). The alloys without intermetallic phases and with minimum porosity have better mechanical properties [13]. A very precise, homogeneous alloy with good mechanical properties is obtained by laying one layer of the alloy powder over another, as confirmed by various authors (Meacock and Vilar [18], Castillo-Oyagüe et al. [19]).

Mechanical properties of the Co-Cr alloy obtained by applying the SLM technology, which are most commonly described, are the following: properties determined based on stress–strain diagram and the metal-ceramic bond strength. The main purpose of metal sintering is to obtain the metal with the highest possible density [20]. Metal density depends on the temperature of the thermally treated metal and the amount of energy required for melting metal powder on one side and scanning, laser power, and the thickness of the powder layer and the thermally treated region on the other.

Jevremović et al. [21] and Zhou et al. [22] demonstrated that sintered and thermally treated Co-Cr alloys show a significantly higher tensile strength and greater modulus of elasticity than cast Co-Cr alloys. Unlike these authors, Lu et al. [23] demonstrated that the density, hardness, and electrochemical properties of the compensation do not depend on the technique applied and that both types meet the requirements of the ISO 22764:2006 standard.

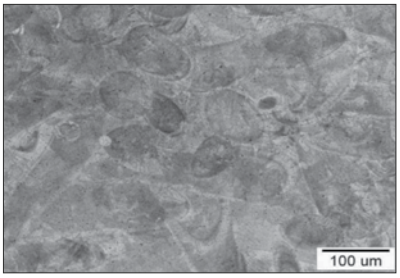

Figure 3. Metallographic microscope micrograph of the sintered Co-Cr alloy surface

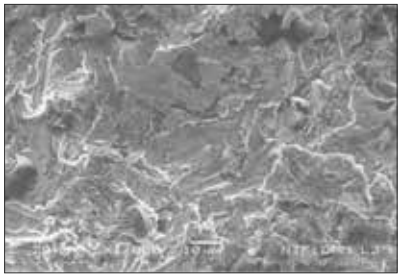

Figure 4. Scanning electron microscope micrograph of the sintered and thermally treated Co-Cr alloy surface

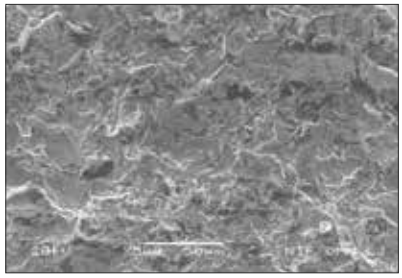

Figure 5. Scanning electron microscope micrograph of the sintered, thermally treated, and sandblasted Co-Cr alloy surface

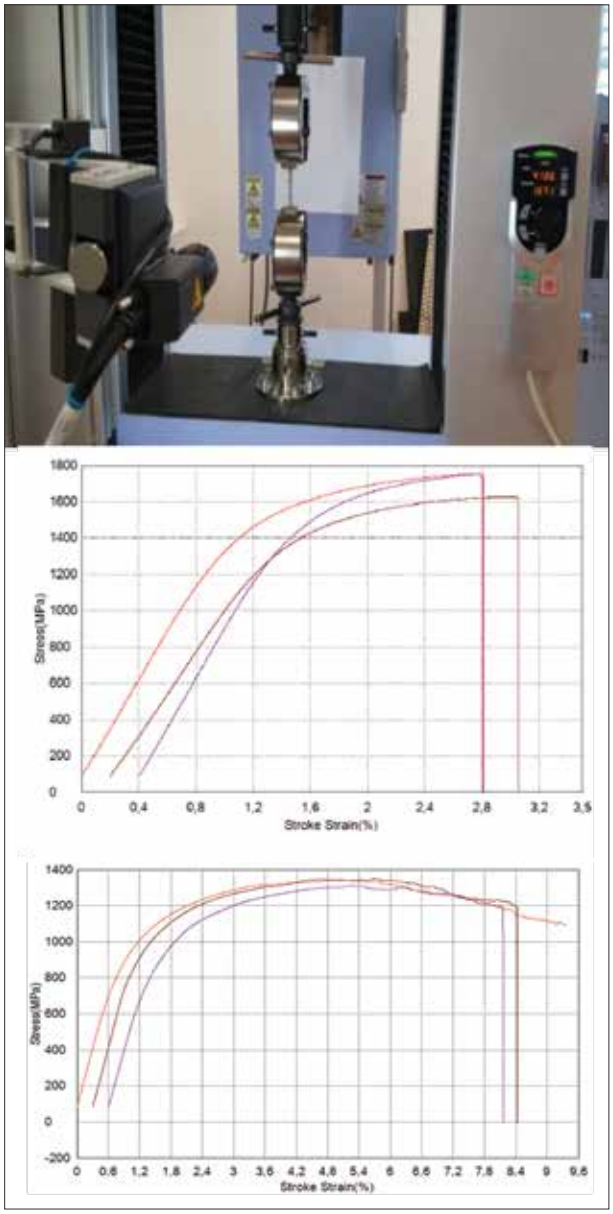

Figure 6. Universal testing machine (A) and stress–strain diagrams for the Co-Cr alloy after sintering (B) and after sintering and thermal treating at 800°C (C)

Residual stress appears as a result of thermally treated individual layers of the melted metal powder. Quick heating is accompanied by quick cooling, which leads to metal expansion, followed by the shrinkage of the metal. This is most striking immediately after the removal of the alloy from the machine, and it is remedied by releasing the residual stress, i.e. by thermal treatment of the alloy. For the purpose of our research, thermal treatment (releasing residual stress) is conducted in the furnace, first at the temperature of 450°C (45 minutes) and then at the temperature of 750°C (60 minutes). After the expiration of the 60-minute period, the furnace is turned off, and the furnace door is opened at the temperature of 600°C, only to turn off the stream of protective gas (argon) at the temperature of 300°C.

Sintered Co-Cr alloy shows higher hardness compared to the same cast alloy. Relevant data found in the literature indicate that the hardness of sintered dental Co-Cr alloys ranges 440–475 HV10, i.e. 382 HV10, whereas the hardness of the cast Co-Cr alloy ranges 325–374 HV10 [24, 25, 26]. Higher hardness and more homogeneous microstructure result in increased corrosion and wear resistance [24]. Subsequent thermal treating of the sintered alloy during the process of baking ceramics (in case of metal-ceramic restorations) does not affect its corrosion resistance [26].

Relevant data found in the literature indicate that the average surface roughness (the profile roughness parameter) immediately after sintering is about 8 μm [27]. After sand-blasting Al_2O_3, the roughness is reduced due to surface homogenization and uniformization. The roughness of the sintered Co-Cr alloy surfaces is several times greater than the roughness of the cast alloy surfaces. This may cause a problem when making mobile restorations (e.g. removable partial denture framework). On the other hand, a rough surface increases the wettability and reduces the contact angle, which enhances the bond between the metal and the ceramics [28].

The SLM technology for the Co-Cr alloy, as a piece in a mosaic, perfectly fits into the technological process automation in smart dental laboratories. This technology uses cyber-physical systems, the Internet, and cloud computing as its platform. In combination with diagnostic information (3D imaging, intraoral scans, etc.) and treatment plan, digital impression and simulation in a virtual articulator, the SLM technology represents a major step towards automation in patient diagnostics and therapy, a major step towards the fourth industrial revolution – Dentistry 4.0. In addition to the already described advantages of sintered alloys in terms of their microstructure and physical and mechanical properties, this technology also ensures significant time saving (dentist's time, patient's time, lab time).

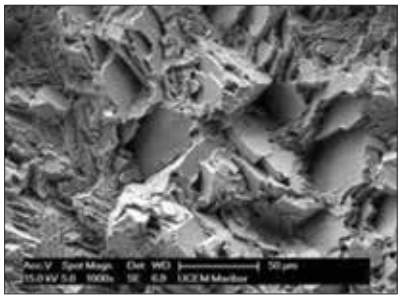

Figure 7. Scanning electron microscope micrograph of a fractured sintered Co-Cr alloy tube surface after mechanical testing

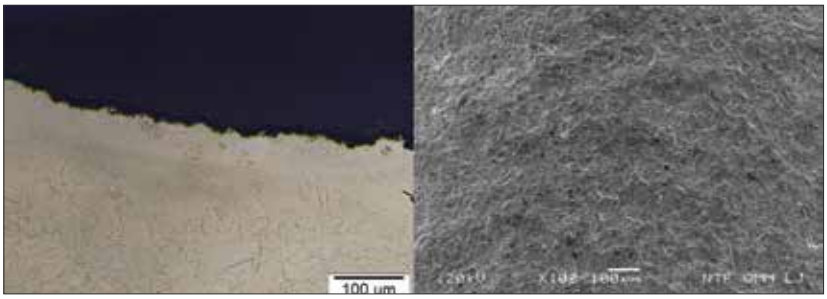

Figure 8. View of the surface (A) and cross-section (B) through the edge of the selective laser melting sinter Co-Cr alloy

Time is money, thereby meaning cheaper diagnostics and therapy. Another significant advantage of the SLM technology lies in the fact that it is an eco-friendly technology (smaller quantities of medical and other waste).

CONCLUSION

Selective laser melting of the Co-Cr alloy is a good example of new technologies based on digitization. Together with other digitized procedures (digital impression, designing virtual restoration, 3D printing), this technology is leading us towards Dentistry 4.0.

1. The qualitative composition of sintered Co-Cr alloys is the same as cast Co-Cr alloys. However, there are certain differences in the quantitative composition of the alloys (higher values for W, Si, and O in the sintered Co-Cr alloys).
2. The microstructure of the sintered Co-Cr alloy is lamellar in nature, with two dominant phases: ε-Co and/or ε-Cr (fcc – face-centered cubic) and γ-Co (hcp – hexagonal close-packed).
3. Mechanical properties of the sintered Co-Cr alloy, prior to thermal treatment, indicate that tested specimens are significantly more brittle compared to the cast Co-Cr alloy. However, after thermal treatments, physical and mechanical properties are approximately the same or superior.
4. The SLM technology has the following advantages over the conventional technology of casting Co-Cr alloy structures: precise metal framework fitting; digital impression and designing virtual restoration, which ensure avoiding mistakes that can occur due to shrinkage of the impression material, the expansion of the plaster that the working model is made of, the expansion of the refractory cast and the shrinkage of the casting upon cooling; eco-friendly technology.

Conflict of interest: None declared.

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Селективно ласерско топљење и синтеровање денталне легуре кобалт-хром

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САЖЕТАК

Увод/Циљ Циљ рада је описати микроструктуру и механичке карактеристике синтероване легуре *Co-Cr* и истаћи њене предности и мане у односу на микроструктуру и механичке карактеристике ливене легуре *Co-Cr*.

Методе У истраживању је коришћена базна легура *Co-Cr, Eosint M EOS Co-Cr SP2* (*EOS GmbH*, Минхен, Немачка) за синтеровање металних конструкција металокерамичких надокнада. Синтеровање метала је обављено на апарату *EOSint M* 280 у струји неутралног гаса аргона. Након тога легура је жарена 20 минута на температури од 800°*C*. Хемијски састав легуре одређиван је енергодисперзивном спектроскопијом. Микроструктура испитиваних узорака легуре посматрана је на оптичком металографском и електронском скенирајућем микроскопу. Физичко-механичке карактеристике мерене су на универзалној кидалици. Узорци су припремани према стандарду *ISO* 527-1:1993.

Резултати Хемијски састав узорака синтероване легуре *Co-Cr* показао је исти квалитативан али различит квантитативан састав у односу на легуре *Co-Cr* за ливење. Микроструктура синтероване легуре *Co-Cr* је ламеларне природе, у којој доминирају две фазе: ε-*Co* и/или ε-*Cr* (*fcc – face-centred cubic*) и γ-*Co* (*hcp – hexagonal close-packed*). У поређењу са ливеном легуром *Co-Cr*, механичке карактеристике синтероване легуре *Co-Cr* су боље или приближно исте.

Закључак Селективно ласерско топљење легуре *Co-Cr* је добар пример нових технологија заснованих на дигитализацији. Заједно са другим дигитализованим процедурама које претходе, ова технологија је предворје новој ери у стоматологији, популарно званој *Dentistry* 4.0. Предности технологије селективног ласерског топљења у односу на технологију конвенционалног ливења металних конструкција од легуре *Co-Cr* су прецизност налегања металне конструкције и чиста технологија.

Кључне речи: селективно ласерско топљење; синтеровање метала; легура *Co-Cr*

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison between Steiner cephalometric and modified Andrews photometric method for assessing antero-posterior position of the maxillary central incisors

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SUMMARY
Introduction/Objective Maxillary incisors, when exposed during smile, are one of the most important facial features. In an attempt to overcome limitations of standard cephalometric methods, Andrews described an approach to determine ideal anteroposterior (AP) position of maxillary central incisors in smiling profile in relation to the forehead. We compared traditional Steiner cephalometric method, using surrounding skeletal landmarks, to the method proposed by Andrews, with the aim of determining whether distant but very noticeable craniofacial structures can affect our impression of tooth position.
Methods The study comprised 90 randomly selected lateral cephalograms, divided into three groups according to maxillary central incisors AP position according to Steiner cephalometric norms. The AP relationship of the maxillary central incisors was measured as a perpendicular distance from facial axis point to the nasion A line and to the vertical line through forehead facial axis point respectively. Student's t-test and Pearson's correlation were used to compare tested variables.
Results There was statistically significant difference between two methods ($p = 0.01108$). According to the Steiner method 46.67% subjects had retrusive incisors and 53.33% subjects had protrusion. Andrews's method showed different results; 35.56% subjects had retrusion, while 64.4% had protrusion.
Conclusion The method proposed by Andrews showed consistently more protrusion than the traditional cephalometric method according to Steiner. Slightly retruded position of maxillary central incisors according to Steiner analysis does not always imply poor facial esthetics, if they have favorable position to the forehead. Low levels of correlation indicate that we should never rely on just one set of parameters.
Keywords: incisors; forehead; facial esthetics

INTRODUCTION

The smile and facial esthetics are the most important motivating factors for many patients to seek orthodontic care. For that reason, most of them are moved solely by a desire to improve appearance, without considering other morphological or functional disorders.

On the other hand, most orthodontic professionals choose their decisions and plan treatment by obtaining optimal occlusal relationship. The literature we found contains numerous studies that have shown significant improvements of post treatment dentofacial features and a high ability of different orthodontic treatments in manipulation of facial attractiveness [1–8]. However, there is also clear evidence that an ideal occlusion often results in a not-so-desirable appearance and facial esthetics [9]. An orthodontic treatment that adheres strictly to cephalometric standards, based on traditional osseous landmarks to define jaw and teeth positions can often be deceiving, since a good facial harmony has been shown to exist within a wide range of cephalometric values. Recently, there has been a paradigm shift that emphasizes the importance of considering the dentition, especially incisors, as a part of the face and not just some cephalometric value among other bony structures [10, 11].

When exposed during smile, maxillary incisors are one of the most important facial features. Most traditional cephalometric values estimate incisors anteroposterior (AP) position relative to surrounding bone structures, like jaw axis, or anterior point of cranial base. Others use soft tissue analysis, like nasolabial angle and E–line that indirectly convey the position of incisors. However, other nearby structures (nose, chin, and forehead) can sometimes distort our perception, visually improving or deteriorating their appearance, thus making traditional hard tissue cephalometric values unreliable. Recently, smile esthetics, especially from the frontal perspective, has frequently been studied [12–15]. In profile, conversely, the maxillary incisors are not typically assessed in relation to other external facial landmarks. In an attempt to overcome aforementioned limitations of standard cephalometric methods, Andrews and Andrews [16] in Six Elements of Orofacial Harmony™, described an approach for determining the ideal AP position of maxillary central incisors in smiling profile, which optimizes

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esthetics of the soft tissue profile. Andrews favors the forehead as a stable landmark because, unlike internal osseous radiographic landmarks, it is a part of the face, with predictable and repeatable relationship to the incisors. Moreover, both lay people and professionals are sensitive to the incorrect AP relationship of the maxillary incisors to the forehead, thus this is a method unconsciously used in determining profile acceptance [17, 18, 19].

The aim of this study was to evaluate and compare traditional Steiner cephalometric method for assessing maxillary central incisors AP position, using surrounding skeletal (osseous) landmarks to the method proposed by Andrews [20], which we modified to use patient radiographs instead of photographs to determine the position of the incisors relative to the forehead.

METHODS

Ninety randomly selected patients (41 males, 49 females, mean age 14.1 years) comprised the study sample. All patients were treated at the Clinic of Dentistry, Faculty of Medicine, University of Novi Sad. Patients with severe congenital skeletal malformations were excluded from the research (clefts, syndromes, etc.). Initial digital cephalometric radiographs were taken, following a standardized procedure, and the hairline was marked with radiocontrast material (barium paste), in order to make point Trichion clearly visible. Radiographs were digitally traced, using Onyx-Ceph 3D (ONYXCEPH^{3}™, Chemnitz, Germany) cephalometric software, and six skeletal and soft tissue landmarks identified. Skeletal landmarks were detected according to Steiner [nasion, A (NA) point, u1FA maxillary central incisor facial axis (FA) point], while landmark points for the forehead were identified as described by Andrews [Trichion, Superion, Glabella, and the forehead facial axis (FFA) point] (Figure 1) [16]. Originally, Andrews's method of evaluation of orofacial harmony is done on lateral photographs, instead, we proposed a radiological evaluation method, on lateral cephalograms, in order to simplify the procedure and avoid any possible problems and inaccuracy due to different head positions and size ratios of photographs and cephalograms.

The entire sample was divided into three groups according to the accepted Steiner analysis cephalometric norms for maxillary central incisors anteroposterior position: group I (norm position u1-NA 2–4 mm), group II (retruded u1-NA < 2 mm) and group III (protruded u1-NA > 4 mm).

In addition to conventional cephalometric nasion-point A line, two vertical reference lines were also constructed: line 1 through the FFA point, line 2 through the maxillary central incisors FA point. The AP relationship of the maxillary central incisors was measured as a perpendicular distance from FA point to the NA line and to the vertical line through forehead's FFA point respectively (Figure 2). Accepted cephalometric norm for the distance of u1FA point to the NA line was 4 mm, as suggested by Steiner, and was assumed to be "u1-NA Δ 4 mm = 0" or base value. A positive value was assigned when u1FA to NA line distance was more than 4mm and negative when less. Base value (0) for the incisors position in relation to the forehead was with u1FA point touching the FFA vertical. A positive value was assigned when maxillary central incisors were anterior to the forehead's FFA point (line1) and negative when posterior.

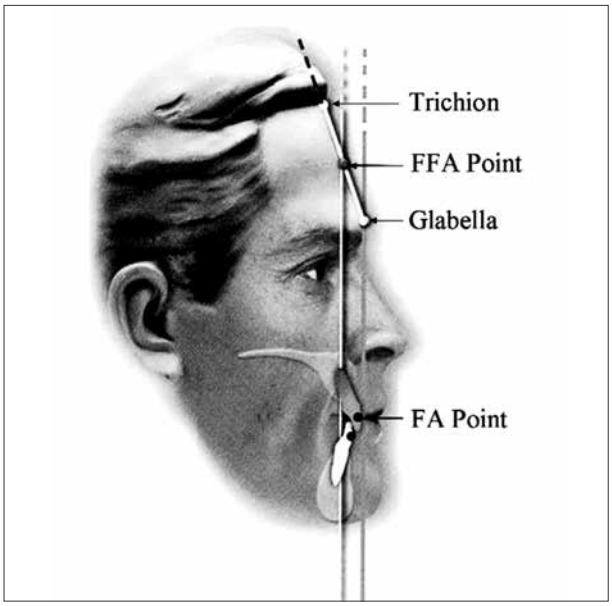

Figure 1. Landmarks used by Andrews to assess the anteroposterior position of the maxillary central incisors relative to forehead [20]

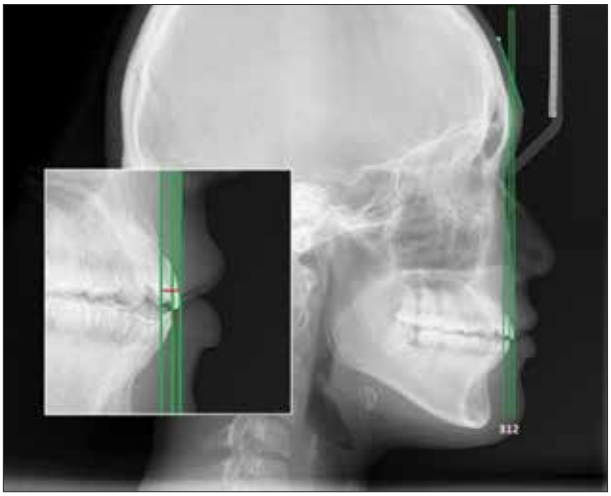

Figure 2. Referent lines on lateral cephalogram used to assess anteroposterior position of maxillary central incisors according to Steiner method and Andrews method;

Line 1 – vertical through the forehead facial axis point; line 2 – vertical through maxillary central incisors facial axis point; line 3 – nasion-point A; (the anteroposterior relationship of the maxillary central incisors was measured as a perpendicular distance from facial axis point to the nasion point A line and to the vertical line through forehead facial axis point, respectively)

Reliability

The reliability of the visual assessment of the morphological characteristics of the forehead was determined by interobserver evaluations between the authors, showed very good agreement (κ = 0.82) as assessed by the kappa coefficient [21].

Duplicate determinations were also carried out for all variables. The measurements were undertaken two weeks apart by the same examiner on a random sample of 20 cephalograms. The systemic error between two measurements was calculated using a paired t-test, for $p < 0.05$, and no significant differences were found for any of the hard or soft tissue variables in the two data sets. The error variance was calculated according to Dahlberg formula.

Data analysis

Descriptive and comparative statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) computer software. The means for both tested values were compared using Student's t-test. P-value of 0.05 or less indicated significant differences. Correlation between variables was tested using Pearson's correlation.

Ethics

The study was conducted according to the Declaration of Helsinki. The study has been approved by the Ethics Committee of the Dentistry Clinic of Vojvodina (Nr: 01-33/2-2019, 29.01.2019).

RESULTS

There were no significant differences between male and female subjects, therefore all data was unified. Descriptive statistics and Student's t-test results of the maxillary central incisors position for the entire sample are shown in Table 1. Relative to the NA point line, maxillary central incisor position ranged from -12.5 mm to +5.8 mm, with an average value of 0 mm and standard deviation of 3.7 mm. Relative to FFA line maxillary central incisors position ranged -16–16 mm, with an average value of 1.45 mm and standard deviation of 6.09 mm. There was statistically significant difference between two cephalometric measurements for evaluation of maxillary central incisors position ($p = 0.01108$). Distribution of established incisors positions according to two different methods are shown in Figure 3 and Figure 4. According to the Steiner method, 42 (46.67%) subjects had retrusive maxillary central incisors, positioned behind threshold value line, and 48 (53.33%) subjects had protrusion. Method proposed by Andrews showed different results; 32 (35.56%) subjects had maxillary central incisors FFA point positioned posterior to the forehead's FFA point indicating retrusive position. Fifty-eight (64.4%) subject had maxillary incisors FFA point somewhere at or in front of the FFA line.

Descriptive statistics and difference testing results for three groups of subjects, according to accepted Steiner analysis cephalometric norms are shown in Table 2. Arithmetic mean values for maxillary central incisors position relative to the NA point line for different groups are 0 mm, -4.10 mm and +3.10 mm and relative to FFA line +3.45 mm, -0.30 mm and +1.45 mm, respectively.

Table 1. Anteroposterior position (mm) of the maxillary central incisors relative to nasion-point A line and to the forehead facial axis line for the entire sample

ALL	Mean	SD	Min.	Max.	t-test (p-value)
u1-NA Δ 4 mm	0	3.7	-12.5	5.8	0.01108*
u1-FFA	1.45	6.09	-16	16	

NA – nasion A; FFA – forehead facial axis; u1-NA Δ 4 mm – accepted cephalometric norm for the distance of u1FA point to the NA line was 4 mm, as suggested by Steiner, and was assumed to be "u1-NA Δ 4mm = 0" or base value; u1-FFA – perpendicular distance from facial axis point to the vertical line through forehead's forehead facial axis point; base value (0) for the incisors position in relation to the forehead was with u1FA point touching the forehead facial axis vertical;
*$p < 0.05$;

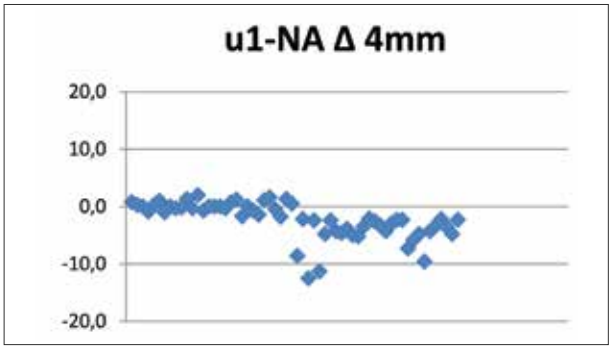

Figure 3. Distribution of established incisors positions relative to nasion-point A line;

u1-NA Δ 4 mm – accepted cephalometric norm for the distance of u1FA point to the nasion-point line was 4 mm, as suggested by Steiner, and was assumed to be "u1-NA Δ 4 mm = 0" or base value

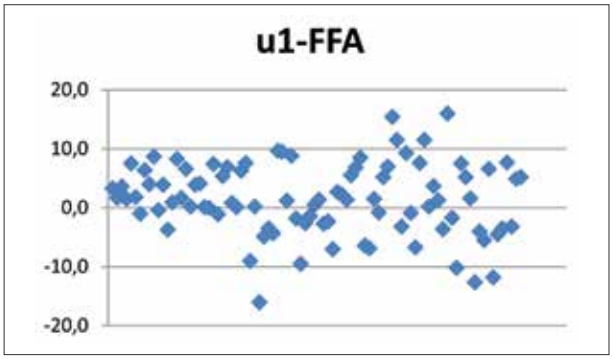

Figure 4. Distribution of established incisors positions relative to the forehead facial axis;

u1-FFA – perpendicular distance from facial axis point to the vertical line through forehead facial axis point; base value (0) for the incisors position in relation to the forehead was with u1FA point touching the forehead facial axis vertical

Significant difference was established for subjects with normo position ($p = 0.00000$) or retruded ($p = 0.00132$) maxillary central incisors.

There was no significant correlation between tested variables overall ($r = 0.24844$), nor in all three groups (Table 4).

DISCUSSION

Of all the factors related to a balanced facial expression and smile esthetics, AP position of the maxillary incisors is one

Table 2. Anteroposterior position (mm) of the maxillary central incisors relative to nasion-point A line and to the forehead facial axis line for three groups (normal, retruded and protruded incisors) according to Steiner cephalometric analyses

Normal (2–4 mm)	Mean	SD	Min.	Max.	t-test (p-value)
u1-NA Δ 4 mm	0	0.96	-1.8	2	0.00000***
u1-FFA	3.45	3.32	-3.7	8.7	
Retruded (< 2 mm)	Mean	SD	Min.	Max.	t-test (p-value)
u1-NA Δ 4 mm	-4.1	2.72	-12.5	-2.1	0.00132**
u1-FFA	-0.3	6.15	-16	9.7	
Protruded (> 4 mm)	Mean	SD	Min.	Max.	t-test (p-value)
u1-NA Δ 4 mm	3.1	1.09	2.1	5.8	0.49020
u1-FFA	1.45	7.62	-12.6	16	

NA – nasion A; FFA – forehead facial axis; u1-NA Δ 4 mm – accepted cephalometric norm for the distance of u1FA point to the NA line was 4 mm, as suggested by Steiner, and was assumed to be "u1-NA Δ 4 mm = 0" or base value; u1-FFA – perpendicular distance from facial axis point to the vertical line through forehead's forehead facial axis point; base value (0) for the incisors position in relation to the forehead was with u1FA point touching the forehead facial axis vertical;
**p < 0.01;
***p < 0.001

Table 3. Percentage of patents with protrusive or retrusive maxillary central incisors relative to nasion-point A line and to the forehead facial axis line

Protrusion		Retrusion
ALL		
46.67%	< u1-NA Δ 4 mm >	53.33%
35.56%	< FFA >	64.4%
Group 1		
33.33%	< u1-NA Δ 4 mm >	66,66%
13.33%	< FFA >	86,67%
Group 2		
0%	< u1-NA Δ 4 mm >	100%
50%	< FFA >	50%
Group 3		
100%	< u1-NA Δ 4 mm >	0%
43.33%	< FFA >	56.67%

FFA – forehead facial axis

Table 4. Correlation between Incisors position relative to nasion-point A line (u1-NA) and to the forehead facial axis (u1-FFA) line

	u1-NA Δ 4 mm	u1-FFA
u1-NA Δ 4 mm	1	
u1-FFA	0.248447	1

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

that can easily be controlled and influenced by orthodontic treatment. If we consider maxillary incisors as a part of the face, then evaluating its position should unavoidably include other facial landmarks. Some facial features such as the nose and chin are very variable and can change considerably over time. Moreover, in many cases, several still widely used cephalometric indices, like nasolabial angle, lip prominence and esthetic lines, does not reflect true position of the maxillary incisors and often depend more on the soft tissue thickness and muscle tonus rather than incisors AP position [22–25].

This research showed a significant difference between maxillary central incisors AP position established by the widely used method according to Steiner, and method by Andrews [16] and Andrews [20] suggesting that the maxillary central incisors should be positioned somewhere at or between the forehead's FFA point and glabella. Average value of u1-NA Δ 4 mm for the entire sample was 0 mm, indicating optimal AP position of maxillary incisors to the NA line, while u1-FFA mean was showing more protruded appearance, but still quite harmonious. Andrews's method showed more subjects with some degree of protrusion, than method according to Steiner. The differences were statistically significant. According to these cephalometric variables, we can conclude that the average patient from tested population is in general with neutral AP position towards a slight protrusion of maxillary central incisors.

If we consider only subjects with harmonious position of maxillary central incisors according to Steiner (group 1) (Table 2), the difference between average values of two indices is much larger.

That inconsistency is even more pronounced in group 2, where all subjects had retruded maxillary central incisors according to the Steiner method, while Andrews's approach showed only one-half of subjects with that characteristic. The average position of maxillary central incisors was far behind NA line, whereas the mean value of u1-FFA variable indicates very harmonious and esthetically pleasing position of incisors in relation to the forehead, as suggested by Andrews that the maxillary central incisors be positioned somewhere at or between the forehead's FFA point and glabella [20]. The established difference was highly significant. Because of these findings, it is evident that the Steiner method is significantly biased towards diagnosing more retrusive maxillary central incisors than photometric method for assessing facial and smile harmony proposed by Andrews.

Even though many studies of facial attractiveness indicate very low acceptance for retrusion of upper incisors, slightly retruded maxillary incisors according to the Steiner analysis, at the beginning or at the end of the treatment, does not always imply poor facial esthetics, if they have a favorable position to the forehead [3, 11, 26, 27]. This finding is emphasizing the importance of using extraoral reference points in evaluating and setting positional treatment goals for upper incisors, since this is the method that the society unconsciously uses to determine facial attractiveness and profile acceptance, rather than, for them obscured, skeletal structures [20, 28].

In group 3 (subjects with protruded incisors according to Steiner method) average value of u1-FFA was showing less protrusive characteristics of central maxillary incisors, than Steiner's method, but the difference was not statistically significant.

A very low level of correlation between compared variables point out that we must never only rely on one set of parameters, and should always incorporate into the assessment more cephalometric, photometric and clinical indices for evaluating the smile, prior to final decisions.

The finding of this study implies that morphology of the face and smile esthetics can sometimes be very deceptive and elusive, and it confirms other authors results that it is possible to obtain harmonious and attractive facial appearance even if some skeletal and dentoalveolar features are deviating from the established norms [27, 29]. Chasing cephalometric norms, without considering the broader view, can sometimes have detrimental effect on facial esthetics. Holdaway [30] in his article concluded that patients for whom orthodontic treatment adhered only to cephalometric standards often did not meet the esthetic principles. Each individual is a unique entity, therefore cephalometric norms for maxillary central incisors AP position should be used only as a general guide and a compliment to visual evaluation of facial attractiveness. As facial esthetics becomes more and more important objective in orthodontics, some of traditional cephalometric dentofacial norms should be evaluated cautiously, or possibly revised, in order to obtain optimal and balanced smile for patients.

CONCLUSION

In general, the method proposed by Andrews and Andrews, for assessing AP position of the maxillary central incisors in relation to the forehead, showed consistently more protrusion than traditional cephalometric method according to Steiner.

Slightly retruded position of maxillary central incisors according to Steiner analysis does not always imply poor facial esthetics, if they have favorable position to the forehead.

Conflict of interest: None declared.

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Поређење Стајнерове кефалометријске и модификоване Ендрузове фотометријске методе за процену антеропостериорног положаја максиларних централних секутића

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САЖЕТАК

Увод/Циљ Максиларни секутићи који се виде током осмеха представљају једну од најважнијих карактеристика лица. У покушају да превазиђе ограничења стандардних рендгенкефалометријских метода, Ендруз предлаже методу за одређивање идеалног антеропостериорног (АП) положаја горњих централних секутића у односу на чело. Стога смо упоредили традиционалну Стајнерову рендгенкефалометријску методу за процену положаја горњих централних секутића, која користи околне скелетне структуре и методу коју је предложио Ендруз, са циљем да се утврди да ли удаљене али веома уочљиве краниофацијалне структуре могу утицати на наш утисак о положају зуба.

Методе Материјал за ову студију састојао се од 90 насумично одабраних латералних рендгенкефалограма, подељених у три групе, у односу на АП позицију максиларних централних инцизива према Стајнеровим рендгенкефалометријским нормама. АП однос максиларних централних секутића је мерен као перпендикуларно растојање од најантериорније тачке крунице горњег централног секутића до линије која спаја тачке *назион* и *субспинале*, као и до вертикалне линије кроз средишњу тачку чела. Студентов *t*-тест и Пирсонова корелација коришћени су за поређење тестираних варијабли.

Резултати Утврђена је статистички значајна разлика између испитиваних метода ($p = 0,01108$). Према Стајнеровој методи, 46,67% испитаника је имало ретрузију секутића, а 53,33% испитаника имало је протрузију. Ендрузова метода је показала другачије резултате: 35,56% испитаника је имало ретрузију, док је 64,40% имало протрузију.

Закључак Метода коју предлаже Ендруз показала је знатно више особа са протрузијом горњих централних секутића него традиционална рендгенкефалометријска метода према Стајнеру. Благо ретрудирани положај горњих централних секутића према Стајнеровој анализи не значи увек и лошу естетику лица, ако секутићи имају повољан положај према челу. Низак ниво корелације указује на то да се никада не треба ослањати на само једну групу показатеља.

Кључне речи: секутићи; чело; естетика лица

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of fibrinolytic therapy on 30-day outcome in patients with intermediate risk pulmonary embolism – propensity score-adjusted analysis

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SUMMARY

Introduction/Objective Patients with submassive (intermediate risk) pulmonary embolism (PE) represent a very heterogeneous group, whose therapeutic strategy still questions whether some groups of patients would have net clinical benefit from fibrinolytic therapy (FT).

Methods From the institutional pulmonary embolism registry, 116 patients with submassive PE were identified, and the relation of their outcome to FT was analyzed using the propensity score (PS) adjustment. The primary endpoint was the composite of death, in-hospital cardiopulmonary deterioration, or recurrence of PE. Safety outcomes were updated TIMI non-CABG related major and minor bleeding.

Results According to Cox regression analysis, the incidence of composite endpoint was significantly lower in patients treated with FT compared to anticoagulant therapy (AT) only (PS adjusted HR 0.22; 95% CI 0.05–0.89; p = 0.039). But, when patients were stratified into four PS quartiles, only patients in the highest PS quartile that received fibrinolysis, had significantly lower composite event rate than patients treated with AT (HR 0.20; 95% CI 0.01–0.56; p = 0.016). The overall mortality of the study group was 5.2% and there was no significant difference between the treatment groups. Total bleeding was significantly more frequent in FT patients (HR 3.07; 95% CI 1.02–13.29; p = 0.047), but not the major one.

Conclusion The use of FT was associated with a better outcome compared to AT in patients with submassive PE, but the benefit was mainly driven from those with highest values of PS, i.e. with the highest baseline risk. The rate of major bleeding was not significantly increased by FT.

Keywords: pulmonary embolism; intermediate risk; fibrinolytic therapy; propensity score

INTRODUCTION

Patients with submassive or intermediate-risk from pulmonary embolism (PE) represent a very heterogeneous group with large variations, both in terms of presentation and prognosis [1, 2]. According to large older registries, Management Strategy and Prognosis of Pulmonary Embolism Registry and International Cooperative Pulmonary Embolism Registry (ICOPER), their mortality ranged 9.6–15.1% [3, 4] and according to recent meta-analysis of randomized trials and newer the Computerized Registry of Patients with Venous Thromboembolism (RIETE) registry data 2–5% [5, 6, 7]. Despite the fact that clinical scores *do* predict adverse outcomes in acute PE [8, 9] and that hybrid studies demonstrate that combinations of right ventricular (RV) dysfunction and elevated biomarkers indicate adverse prognosis [10, 11], still *management of submassive PE crosses the zone of equipoise* [1]. The latest European guidelines are more precise, in terms of not recommending routine use of primary systemic thrombolysis in patients not suffering from shock or hypotension, but on the other hand, they strongly suggest close monitoring in patients with intermediate-high risk PE to permit early detection of hemodynamic decompensation or circulatory collapse, and timely initiation of rescue reperfusion therapy. In addition, set-up of multidisciplinary teams for management of selected cases of intermediate-risk PE should be considered [2].

Although no study has yet shown clear benefits of fibrinolytic therapy (FT) in terms of overall survival in patients with intermediate-risk PE, there are data indicating beneficial effects on other important short and long-term outcomes [12, 13, 14]. Randomized, prospective studies have found beneficial effect of FT on clinical outcomes, although in the largest one, at the expense of increased risk of major and intracranial bleeding [15, 16, 17]. Therefore, the main unresolved issue remains whether specific group of patients with intermediate risk PE would profit from FT, without increasing the risk of serious adverse events.

The ideal design of the study investigating treatment effects implies random allocation of patients to different types of therapy. However, these studies may have limited applicability, and their conclusions often correspond less to everyday clinical practice than those from

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observational, registry studies. On the other hand, registry data usually have significant baseline between-group differences in observed covariates, which need to be adjusted by adequate statistical methods. Therefore, based on clinical and echocardiographic criteria, we designed this observational registry study, in order to investigate the propensity score-adjusted association of FT and 30-days outcome in patients with acute submassive/intermediate risk PE.

METHODS

This is a single center, investigator initiated, observational cohort study, conducted in a tertiary, academic teaching hospital, from January 2011 to May 2014**.** The diagnosis of acute submassive/intermediate risk PE was made as follows: typical symptoms and signs of PE lasting no more than seven days, without systemic hypotension at the admission (i.e. with systolic blood pressure > 90 mmHg and absence of a drop of systolic blood pressure by ≥ 40 mmHg compared to standard values, lasting > 15 minutes), but with RV dysfunction confirmed by echocardiography. The criteria for RV dysfunction were [18]:

1. RV end-diastolic diameter > 3 cm in parasternal long axis and right/left ventricular end-diastolic diameter ratio > 0.6 (both in the absence of RV hypertrophy);
2. hypokinesia of RV-free wall in any view and/or Mc Connell sign;
3. tricuspid regurgitant jet velocity 2.5–3.5 m/s in the absence of inferior vena cava collapse on inspiration and/or estimated RV systolic pressure 40–60 mmHg. Patients had to fulfill at least one from each of three above-mentioned criteria. The diagnosis of PE was confirmed either by multidetector computed tomography scan, when available (n = 68), or by ventilation/perfusion mismatch on lung scintigraphy scan (n = 48). Contraindications to fibrinolysis were any prior intracranial hemorrhage, known structural or malignant intracranial disease, ischemic stroke within six months, gastrointestinal bleeding within the last month, uncontrolled hypertension (systolic blood pressure > 180 mmHg), any active bleeding, or known bleeding diathesis, recent major trauma/surgery/head injury in the preceding three weeks.

The study protocol was approved by the University of Belgrade, Faculty of Medicine Ethical Committee and done in accordance with the legal standards. Since we used routine data registry, written informed consent was not required, but all patients gave an oral informed consent for the use of their unidentified data and follow-up participation.

Study protocol

All patients included in the study received an intravenous bolus of unfractionated heparin (80 U/kg) and underwent immediate clinical, ECG, echocardiographic, arterial blood gas and D-dimer evaluation, in addition to standard laboratory analysis. Cardiac troponin I (when available) was measured using micro particle enzyme immunoassay – MEIA kit (Abbott Laboratories) and the reference value was < 0.04 ng/mL. Since at the study time, no prognostic score was officially recommended, all further decisions concerning the treatment were made at the discretion of the clinician caring for each patient. If the decision was made for FT, Food and Drug Administration approved protocol for alteplase was applied (10-mg iv bolus, followed by a 90-mg intravenous infusion over a period of two hours), followed by an intravenous infusion of unfractionated heparin after getting an activated partial-thromboplastin time (aPTT) < 70 sec. If the decision was made for anticoagulant therapy only, the infusion of unfractionated heparin was started at a rate of 18 U/kg/h, and the rate was subsequently adjusted to maintain the aPTT at 1.5–2 times the control. Measurements of the aPTT were performed at six-hour intervals on day one and two and at 12-hour intervals thereafter. Overlapping with oral anticoagulant therapy started on hospital day three and was maintained until a therapeutic range of the international normalized ratio (INR 2.5–3) was stable for two days. Patients were examined for symptoms, blood pressure, and heart rate every six hours for the first 48 hours, then every 12 hours until clinical stabilization, and daily up to discharge; Sat O_2 was continuously monitored for the first 48 hours. All patients had complete blood count and fibrinogen concentration measured at enrollment and daily until discharge. On each day, every patient was examined for the occurrence of any bleeding episodes. During hospitalization, color-Doppler scan of the inferior limbs' deep veins was also performed in all the patients.

Outcomes

Primary outcome of the study was the composite of death, cardiopulmonary deterioration, or recurrence of PE (nonfatal) within 30 days of presentation. Cardiopulmonary deterioration was defined as:

1. the need for cardiopulmonary resuscitation or any spontaneous episode of hypotension (drop of systolic blood pressure below 20 mmHg compared to the admission value) with signs of end-organ hypoperfusion;
2. respiratory failure (worsening dyspnea with drop of arterial oxyhemoglobin saturation level below 90%).

Confirmation of recurrent PE was done by appearance of a new perfusion defect by lung scan or multidetector computed tomography in patients having symptoms and signs of recurrent PE attack. Safety outcomes were updated TIMI non-CABG related major and minor bleeding [19].

Statistical analysis

Continuous variables were expressed as means ± standard deviation or medians with interquartile ranges, depending on their type of distribution, examined by Kolmogorov–Smirnov test. Logistic regression analysis was used for unadjusted (univariate) comparisons of baseline demographic, clinical, electrocardiographic, echocardiographic, and

biochemical characteristics between two groups (patients receiving FT *vs.* patients with anticoagulant therapy only). To control for baseline differences in observed covariates between groups, a propensity score-based approach was applied [20, 21]. We estimated the odds ratios (ORs) of receiving thrombolysis by fitting separate invariable logistic regression models, with each individual covariate as the independent variable. Variables with p-value ≤ 0.1 were selected, and further tested for mutual correlation (Pearson or Spearman correlation, depending on their type of distribution). For those variables with significant and high (> 0.7) correlation, only one was used for building a multiple logistic regression model, that finally gave predicted probabilities (propensity scores) for each patient to receive FT. In order to evaluate the association of FT and 30-days outcomes, propensity score-adjusted Cox regression analysis was performed. Finally, predefined sub-group analysis of patients stratified into four risk groups, according to their PS values (PS quartiles) was done (highest PS quartile – highest risk). For each group, Cox regression analysis was performed, in order to assess the potential heterogeneity of fibrinolytic treatment impact on primary endpoint. Kaplan–Meier method was used to present difference in event-free survival curves of treatment groups in PS quartiles. For all analyses, p-value < 0.05 was considered statistically significant. Statistical analysis was performed with SPSS software Version 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Study population

From the institutional PE registry, 314 patients with acute PE were identified. Sixty-two of them (19.7%) had massive/high risk PE; 120 (38.3%) patients had submassive/intermediate risk PE (in 116 of them, being the first episode of PE), and 132 (42%) patients had non-massive/low risk PE. In all 116 patients included in the study, the post-hoc calculation of simplified PESI score was ≥ 1. Baseline characteristics of the study patients, according to treatment groups, are presented in Table 1. At the beginning of the study, troponin was not routinely measured, so values for the first 35 patients are not available. Analysis of the rest 81 patients showed that patients in the highest PS quartile had significantly higher values of cardiac troponin I compared to those in lower quartiles (quartile IV (1.64 ± 1.10 ng/mL) *vs.* quartiles I (0.15 ± 0.08 ng/mL) and II (0.17 ± 0.10 ng/mL) – p < 0.01; quartile IV *vs.* quartile III (1.02 ± 0.91 ng/mL), p = 0.08).

Treatment

Fibrinolytic therapy was given to 25/116 (21.5%) patients with intermediate risk PE, and only anticoagulant therapy to 91/116 (78.5%) patients. Patients who received thrombolysis had higher incidence of cyanosis at the admission, as well as higher respiration rate, greater end-diastolic diameter of the right ventricle, higher D-dimer values and lower Sat O_2 level. Propensity score was constructed as previously described, and included following variables in multiple logistic regression model: respiration rate, cyanosis, D-dimer value, and Sat O_2 level. After PS-adjustment, treatment groups did not differ in observed covariates (Table 1).

Outcomes

During the first 30 days after admission, primary composite clinical endpoint occurred in 18/116 (15.5%) patients; in 3/25 patients (12%) treated with thrombolysis, and in 15/91 patients (16.5%) treated with heparin only. According to PS-adjusted Cox regression analysis, the rate of primary composite endpoint was significantly lower in patients treated with FT compared to those treated with heparin only (PS-adjusted HR 0.22; 95% CI, 0.05–0.89; p = 0.039) (Table 2). After risk stratification of patients into quartiles according to their PS values, predefined subgroup Cox analysis showed that only patients in the highest PS quartile that received thrombolysis, had significantly lower primary composite event rate compared to patients treated with heparin only (13.3% *vs.* 50%; HR 0.20; 95% CI 0.01–0.56; p = 0.016) (Table 3). Figure 1 shows Kaplan–Meier analysis of event free survival between two treatment groups in the highest risk (PS) quartile. The overall mortality was 5.2%. Individual events did not differ significantly between treatment groups (Table 2).

Total bleeding rate was higher in patients who received thrombolysis (12%, *vs.* 5.5% in the anticoagulant therapy group; PS-adjusted HR 3.07; 95% CI 1.02–13.29; p = 0.047). Major bleeding rate was similar in both groups (4% *vs.* 2.3%, p = 0.643) (Table 2); one patient (in the fibrinolytic group) had non-fatal intracranial bleeding, and no patient had fatal bleeding.

DISCUSSION

The main findings of our study are:

1. Fibrinolytic therapy was associated with lower 30-days PS-adjusted rate of primary composite endpoint (death, cardiopulmonary deterioration, or PE recurrence) in patients with intermediate-risk PE;
2. The observed reduction in primary composite endpoint rate was largely driven by reduction of events in patients with the highest baseline risk (i.e. in patients within the highest PS quartile);
3. Bleeding was more frequent in patients receiving thrombolysis, but not a major or a fatal one.

Our study population was homogenous in terms of the uniform definition of intermediate-risk PE at admission, which was further confirmed by Simplified Pulmonary Embolism Severity Index score ≥ 1 for all patients. Nevertheless, we observed a large heterogeneity in terms of a wide range of important parameters like respiration rate, incidence of cyanosis, D-dimer values, and Sat O_2 level (Table 1), which eventually influenced therapy decision.

Table 1. Baseline characteristics and their unadjusted and propensity score (PS) adjusted comparison between treatment groups

Variable	Heparin (n = 91)	Thrombolysis (n = 25)	Unadjusted OR (95% CI)	Unadjusted p	PS-adjusted OR (95% CI)	PS-adjusted p
Demographics						
Age (years), X ± SD	61 ± 11	59 ± 17	1.01 (0.98–1.04)	0.576	1.01 (0.97–1.05)	0.613
Sex (male) %	41.7	39.1	1.07 (0.43–2.70)	0.881	1.31 (0.43–3.97)	0.630
Previous or concomitant disease						
Previous surgery, %	29.7	26.1	0.84 (0.29–2.35)	0.735	0.78 (0.25–2.57)	0.704
Previous immobilization, %	10.1	17.4	1.71 (0.48–6.03)	0.407	3.26 (0.76–13.88)	0.110
Chronic cardiopulmonary disease, %	21.9	26.1	1.25 (0.44–3.59)	0.675	1.82 (0.52–6.36)	0.349
History of cancer, %	12.1	13	0.90 (0.23–3.46)	0.878	0.66 (0.11–3.85)	0.642
Symptoms						
Dyspnea, %	90.1	95.6	2.41 (0.29–20.09)	0.415	0.70 (0.07–6.55)	0.758
Pleural pain, %	29.5	27.8	0.94 (0.37–2.34)	0.889	1.28 (0.43–3.79)	0.661
Syncope, %	17.3	19.7	1.53 (0.49–4.79)	0.467	1.67 (0.45–6.21)	0.447
Palpitations, %	21.9	26.1	1.25 (0.44–3.59)	0.675	1.51 (0.44–5.14)	0.510
Signs						
Cyanosis, %	7.7	30.4	3.62 (1.12–11.02)	**0.031**	0.44 (0.08–2.47)	0.349
SAP (mm Hg), X ± SD	126.1 ± 21	127.5 ± 23.4	0.99 (0.97–1.02)	0.847	1.01 (0.99–1.04)	0.391
DAP (mm Hg), X ± SD	80.4 ± 12.1	85.5 ± 19.8	1 (0.97–1.04)	0.865	1.02 (0.98–1.06)	0.319
Heart rate, X ± SD	103.1 ± 20.5	107.1 ± 22.4	1.01 (0.98–1.03)	0.340	0.98 (0.95–1.01)	0.184
Respiration rate, med (IQR)	21 (15, 26)	30 (24, 32)	1.14 (1.04–1.24)	**0.004**	1.03 (0.92–1.14)	0.629
Right S3, %	23.1	43.5	2.56 (0.98–6.68)	0.054	0.64 (0.17–2.43)	0.510
Signs of DVT, %	71.6	78.2	1.22 (0.45–3.33)	0.796	1.11 (0.33–3.71)	0.869
Electrocardiographic						
S1Q3T3, %	58.2	52.2	0.78 (0.31–1.96)	0.600	0.39 (0.12–1.24)	0.111
New RBBB, %	13.1	13	0.99 (0.25–3.83)	0.986	0.84 (0.18–3.96)	0.830
Negative T in V1-V3, %	25.2	39.1	1.90 (0.73–4.97)	0.191	2.27 (0.73–7.05)	0.155
Echocardiographic						
EDDRV (cm), X ± SD	3.2 ± 0.45	3.5 ± 0.5	3.01 (1.09–8.29)	**0.033**	1.69 (0.53–5.38)	0.376
EDRV/EDLV, X ± SD	0.8 ± 0.2	0.9 ± 0.2	0.88 (0.03–28.38)	0.578	0.18 (0.01–12.08)	0.427
RVSP (mmHg), X ± SD Mc Connell sign, %	45.4 ± 18.5 76.9	50.2 ± 15.5 91.3	1.01 (0.98–1.05) 1.56 (0.32–7.59)	0.488 0.581	0.99 (0.96–1.04) 0.90 (0.18–5.01)	0.881 0.904
Laboratory						
D-dimer (ng/mL), X ± SD	1647 ± 1524	2690 ± 2152	1.69 (1.11–2.57)	**0.014**	1.18 (0.72–1.71)	0.514
pO_2 (kPa), X ± SD	8.3 ± 1.9	8 ± 3.8	1.11 (0.82–1.51)	0.505	1.22 (0.83–1.79)	0.311
Sat O_2 (%), X ± SD	89.1 ± 5	83.1 ± 7	0.87 (0.80–0.95)	**0.002**	0.98 (0.88–1.09)	0.708

SAP – systolic arterial pressure; DAP – diastolic arterial pressure; DVT – deep vein thrombosis; RBBB – right bundle branch block; EDDRV – end-diastolic dimension of the right ventricle; EDDLV – end-diastolic dimension of the left ventricle; RVSP – right ventricular systolic pressure; Sat O2 – arterial oxyhemoglobin saturation level

Table 2. Propensity score-adjusted comparison of 30-days clinical outcomes in the two treatment groups

Parameters	Heparin (n = 91)	Thrombolysis (n = 25)	PS-adjusted HR (95% CI) for thrombolysis	PS-adjusted p
Primary composite endpoint	15 (16.5%)	3 (12%)	0.22 (0.05–0.89)	0.039
Death	5 (5.5%)	1 (4%)	0.32 (0.03–3.58)	0.352
CP deterioration*†	6 (6.6%)	1 (4%)	0.25 (0.01–1.66)	0.123
Pulmonary embolism recurrence*	4 (4.4%)	1 (4%)	0.71 (0.06–9.06)	0.795
Bleeding (total)	5 (5.5%)	3 (12%)	3.07 (1.02–13.29)	0.047
Major	2 (2.2%)	1 (4%)	1.93 (0.46–26.37)	0.643
Minor	3 (3.3%)	2 (8%)	3.54 (1.41–16.34)	0.049

*Non fatal;
†not related to pulmonary embolism recurrence

Table 3. Distribution of primary composite events in treatment groups across propensity score quartiles

PS quartiles	Therapy	Number of patients	Number of events (%)	PS-adjusted HR (95% CI) for thrombolysis	PS-adjusted p
I (≤ 0.030)	Heparin Thrombolysis Overall	28 1 29	0 0 0	NA	NA
II (0.031–0.089)	Heparin Thrombolysis Overall	26 3 29	4 (15.4) 0 4 (13.8)	NA	NA
III (0.090–0.227)	Heparin Thrombolysis Overall	23 6 29	4 (17.4) 1 (16.7) 5 (17.2)	1.03 (0.11–9.22)	0.982
IV (> 0.227)	Heparin Thrombolysis Overall	14 15 29	7 (50) 2 (13.3) 9 (31)	0.20 (0.01–0.56)	0.016

PS – propensity score

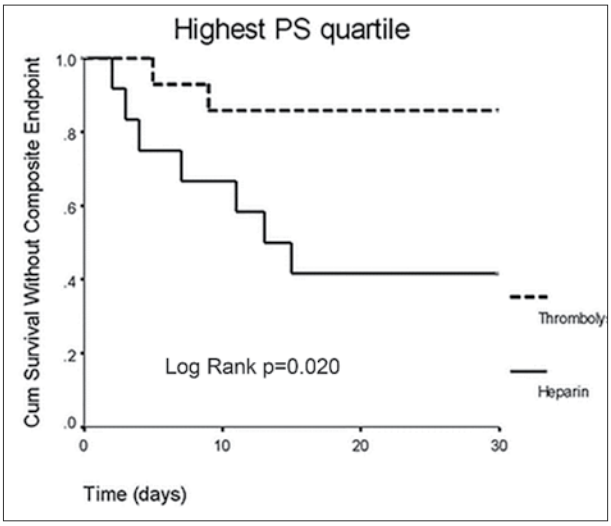

Figure 1. Kaplan–Meier curves of event-free survival in patients in the highest propensity score quartile, according to therapy

Obviously, patients who received FT were "sicker," according to significantly higher values of previously mentioned variables, as well as larger end-diastolic diameter of the right ventricle. Propensity score values were calculated as a chance of receiving thrombolysis for every patient, therefore representing their baseline risk of adverse outcome (highest quartile – highest risk). This was confirmed by the incidence of primary endpoint in different PS quartiles, being 0% in the first, 13.8% in the second, 17.2% in the third, and 31% in the fourth quartile. Similar rate of an adverse 30-days outcome (29.2%) for patients in the highest-risk stratum was found by Bova et al. [22], using a new prognostic model on the basis of four simple variables, in order to ameliorate stratification of patients with intermediate-risk PE.

The benefit of FT in our study was mainly driven by the reduction of events in patients within the highest PS quartile, i.e. with the highest baseline risk, depicted in variables that constituted PS (respiration rate, cyanosis, D-dimer value, and Sat O_2 level). In addition, patients in the highest PS quartile had significantly higher values of cardiac troponin I compared to quartiles I and II, which further supports their higher risk. In such subset of patients, waiting for hemodynamic deterioration to occur could be dangerous, since it can abruptly progress to shock or need for cardiopulmonary resuscitation, which puts them in a significantly higher risk of death and probably less effective "rescue thrombolysis" [23, 24, 25]. Therefore, Kearon et al. [26], in the CHEST guidelines, propose that in patients without hypotension, deterioration in markers such as increased heart rate, a decrease in systolic BP (which remains > 90 mmHg), worsening gas exchange, progressive right heart dysfunction on echocardiography, or an increase in cardiac biomarkers, may also prompt the use of FT. In the remaining three PS quartiles of patients in our study, 11.5% of patients received FT, without any clinical benefit. This confirms the statement that FT should not be considered for less severe forms of intermediate-risk PE.

As expected, total bleeding was more frequent in patients who received thrombolysis; but no major bleeding occurred. Several meta-analyses have evaluated the bleeding risk of FT in intermediate risk PE, most of them showing significant association with major or intracranial bleeding [5, 6, 27, 28]. However, the subgroup analysis based on the type of fibrinolytic agent, by Marti et al. [6], suggested a higher risk for both major and fatal or intracranial hemorrhage in patients treated by tenecteplase compared to those treated with alteplase. The absence of significant difference in major bleeding in our study could probably be a result of the following facts:

1. the mean age of the thrombolysed patients was 59 years (of note, only two patients in that group were over 70 and one of them had non-fatal intracranial bleeding);
2. we used alteplase;
3. heparin was withheld during the alteplase infusion and started after optimal aPTT was obtained;
4. before the initiation of FT meticulous bleeding risk evaluation was done.

Still, the safety issue should be interpreted cautiously, because of the limited sample size, as well as the fact that major bleeding rate in patients with fibrinolysis was almost twice of that with anticoagulant therapy only (though not reaching statistical significance).

Although not designed to evaluate risk stratification, our results support the statement that current risk stratification scheme needs further improvement for intermediate

risk group of patients, in order to optimize better identification of candidates for reperfusion treatment [2, 29]. Further potential benefit could eventually be accomplished by development and incorporation of a new major/intracranial bleeding risk score in PE, like the PE-CH score, proposed by Chatterjee et al. [30].

Study limitations

First, since our study was performed in a single-center acute cardiac care unit, on a relatively small number of patients, selection bias may exist. Second, due to non-randomized allocation of FT, in spite of using PS-adjustment, we could not control for non-observed covariates. Third, patients in our study (especially those who received FT) were younger than in other cohort/registry studies, which makes the extrapolation of the results uncertain. Therefore, our results should prospectively be validated in a larger, randomized trial.

CONCLUSION

We demonstrated that the use of FT was associated with better outcome compared to anticoagulant therapy in patients with the highest baseline risk among submassive/intermediate risk PE patients. Although FT increased the incidence of total bleeding events, the rate of major bleeding in our study was not significantly higher in these patients; still, one patient who received fibrinolysis had non-fatal intracranial bleeding.

NOTE

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Conflict of interest: None declared.

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Утицај фибринолитичке терапије на 30-дневни исход болесника са средњеризичном плућном емболијом – анализа прилагођена „пропензити скором“

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САЖЕТАК

Увод/Циљ Болесници са субмасивном (средњеризичном) плућном емболијом (ПЕ) представљају веома хетерогену групу и код њих још увек постоји дилема у вези са укупном терапијском користи од фибринолитичке терапије (ФТ) у односу на лечење хепарином, код одређених болесника.

Методе Из институционалног регистра плућних емболија идентификовано је 116 болесника са субмасивном ПЕ и анализиран је исход тих болесника у односу на примену ФТ коришћењем пропензити скора (ПС), који је представљао шансу сваког болесника да добије ФТ. Примарни циљ је био композитни догађај – смрт, интрахоспитално кардиопулмонално погоршање и рецидив ПЕ. Безбедносни исход су била ревидирана *TIMI non-CABG related* велика и мала крварења.

Резултати Коксовом регресионом анализом добијена је значајно нижа учесталост композитног циља код болесника лечених ФТ у односу на оне лечене хепарином (ПС прилагођен *HR* 0,22; 95% *CI* 0,5–0,89; $p = 0,039$). Али, када су болесници стратификовани у четири групе, на основу ПС квартила, само болесници из највишег ПС квартила лечени ФТ имали су значајно ређи композитни циљ у односу на оне лечене хепарином (*HR* 0,20; 95% *CI* 0,01–0,56; $p = 0,016$). Укупни морталитет посматране групе је био 5,2% и није било значајне разлике међу групама. Укупна крварења су била чешћа у фибринолитичкој групи (*HR* 3,07; 95% *CI* 1,02–13,29; $p = 0,047$), али не и велика крварења.

Закључак Примена ФТ била је удружена са бољим исходом у односу на лечење хепарином, али је корист доминантно добијена код оних са највишим вредностима ПС, тј. са најтежом клиничком сликом при пријему. Учесталост великих крварења није била значајно повећана применом ФТ.

Кључне речи: средњеризична плућна емболија; фибринолитичка терапија; пропензити скор

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Influence of breastfeeding and timing of gluten introduction on the onset of celiac disease in infants

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SUMMARY

Introduction/Objective The classic type of celiac disease (CD) is most common in children under two years of age.
The aim of this study was to investigate whether breastfeeding, particularly breastfeeding during gluten introduction, and timing of gluten introduction, influence the onset of CD at this age.

Methods We retrospectively analyzed medical records of 93 children, 40 in the first and 53 in the second year, with a classic CD diagnosed at the University Children's Hospital, Belgrade between 2000 and 2010. The diagnosis of CD was based on the criteria of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) from 1989.

Results Duration of breastfeeding reduced the onset of the CD in the first year p = 0.039 (OR = 1.43 95% CI 1.019–1.899). Also, breastfeeding at the time of gluten introduction significantly delayed the age at diagnosis (F = 1.671, t = 2.39, p = 0.029). The timing of gluten introduction did not affect the age of occurrence of CD in these group of children.

Conclusion Longer breastfeeding, and breastfeeding at the time of gluten introduction, postponed the onset of classic CD in patients up to two years. The association between the occurrence of CD and the time of introduction of gluten in this age group of patients has not been established.

Keywords: classic celiac disease; children up to 2 years; breastfeeding; age of gluten introduction

INTRODUCTION

Celiac disease (CD) is an immune-mediated systematic disease caused by the ingestion of gluten that appears in genetically predisposed individuals. The most important genetic factor is the human leukocyte antigen (HLA) locus DQ2 and HLA-DQ8 haplotypes, while the gluten, most important environmental factor, is required to trigger the disease [1, 2]. Other factors may contribute to the pathogenesis and expression of CD, namely additional genetic loci, sex, breastfeeding, timing of gluten introduction, gut microbiota, mode of delivery, metabolic profile of patients, etc. [3]. The disease may be symptomatic, with the occurrence of gastrointestinal and non-gastrointestinal symptoms. In addition, the course of the disease may be asymptomatic. Symptomatic form includes classic and atypical presentation. The classic form of the disease, which primarily occurs in children aged 9–24 months, is characterized by chronic diarrhea, vomiting, abdominal distention, and malnutrition [4].

Some recent randomized controlled trails concluded that there is no influence of timing of gluten introduction on the risk of developing CD [5, 6]. New observational studies showed that breastfeeding, never during gluten introduction, influenced the risk of developing CD [5, 6]. Obviously, there is a need to further clarify the role of environmental factors in pathogenesis of CD.

The aim of this study was to investigate whether breastfeeding, particularly breastfeeding during gluten introduction, and timing of gluten introduction, influenced the onset of CD in infants.

METHODS

We retrospectively analyzed medical records of 93 infants (children up to two years of age; 61 girls and 32 boys), diagnosed with classic form of CD at the University Children's Hospital in Belgrade, between 2000 and 2010. The study protocol was approved by the hospital Ethics Committee. Of the 93 infants, 40 were diagnosed in the first year and 53 in the second, and all of them had severe gastrointestinal symptoms, characterized by a chronic diarrhea, poor

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appetite, and failure to thrive. The diagnosis of CD was based on the criteria of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [7]. In all the participants, the enteropathy was destructive, in 90% it was total or subtotal, and in 10% partial.

In medical records, we analyzed duration of breastfeeding before the diagnosis, timing of gluten introduction, the duration of symptoms, and the age at diagnosis. Having the retrospective study, we did not insist on exclusively breastfeeding, we assumed that the infant had been breastfed if mother had produced enough milk for a minimum of three complete nursing per day.

Participant descriptive statistics are shown in Table 1.

Table 1. Descriptive statistics of 93 infants with classic celiac disease

Age (months) at celiac diagnosis	14.28 ± 4.78
Duration (months) of symptoms	2.08 ± 1.84
Duration (months) of breastfeeding	3.33 ± 3.77
Age (months) at gluten introduction	4.64 ± 1.23

The participants were divided into two groups based on breastfeeding status at the time of gluten introduction. Twenty-four participants were breastfeed at the time of gluten introduction, and 69 were not. In addition, according to new ESPGHAN position paper [8], we divided participants regarding timing of gluten introduction: before the fourth month (n = 12) and after (n = 81).

Binary logistic regression was conducted to estimate whether duration of breastfeeding and timing of gluten introduction influenced the risk of disease occurrence in the first year of life. Differences regarding age at diagnosis between the groups of infants formed based on timing of gluten introduction and breastfeeding during gluten introduction were determined using χ^2 test. For all statistical analyses OpenStat (Bill Miller, Iowa, USA) software for Windows, version 11.9.08 (http://openstat.en.softonic.com/) was used.

RESULTS

Binary logistic regression showed that duration of breastfeeding and timing of gluten introduction influenced the risk of CD occurrence in the first year of life. The model with statistical significance influenced the risk (χ^2 = 16.14; df = 4; p = 0.001), and explained 24.3–32.4% variance. The only variable with significant prediction was duration of breastfeeding, which reduced the onset of the disease in the first year p = 0.039 (OR = 1.43 95%, CI = 1.019–1.899) (Table 2).

Table 2. Binary logistic regression for the analysis of association between duration of breastfeeding and occurrence of celiac disease after the first year in 93 infants diagnosed with classic form of celiac disease

Variable	B	SE	p	Exp (B) or OR	95% CI for OR
Duration of breastfeeding	0.383	0.141	0.010	1.439	1.019–1.899
Constant	-2.369	1.467	0.106	0.094	

OR – odds ratio; CI – confidence interval; SE – standard error

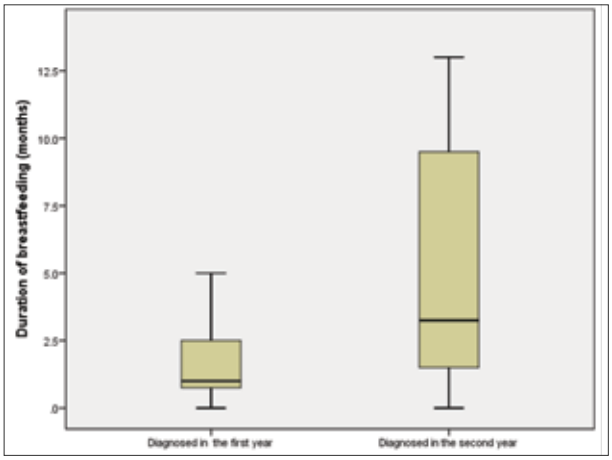

Figure 1. Duration of breastfeeding in infants with classic celiac disease diagnosed in the first and in the second year

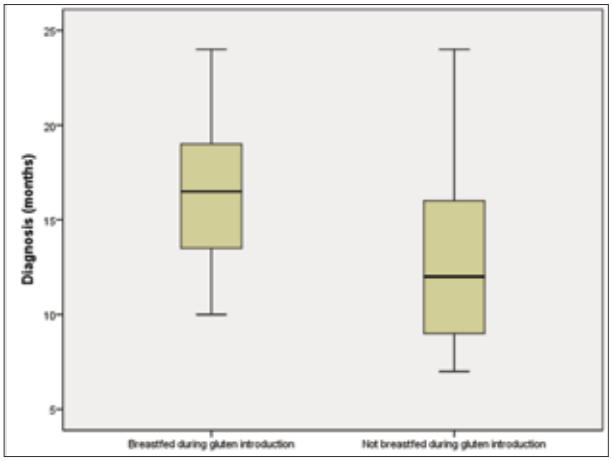

Figure 2. Occurrence of the celiac disease in two groups of infants based on breastfeeding status during gluten introduction

Since the regression model suggested that timing of gluten introduction was not associated with the postponing the diagnosis, we compared the two groups of infants based on the timing of gluten introduction. Although in the first group (gluten introduced prior to the fourth month) the onset of the disease was earlier (12.75 ± 4.15months) compared with the second group (14.69 ± 4.9 months), there was no significant difference (F = 1.036; p = 0.197) between them.

Longer breastfeeding was associated with delayed diagnosis of CD. Infants with the diagnosis made in second year were breastfed for 5.27 ± 4.68 months, while those diagnosed in the first year had shorter duration of breastfeeding (1.63 ± 3.99 months) and the difference between groups was significant (F = 5 6.57, t = -4.15; p < 0.01) (Figure 1).

In the group of infants that were not breastfed at the time of gluten introduction, the mean age at diagnosis was 13.20 ± 5.01, and in the other group it was 16.31 ± 3.99 months, and the age at diagnosis was significantly delayed into second group (F = 1.671, t = 2.39, DF = 91, p = 0.029) (Figure 2).

Also, we found that there was twice as much girls than boys diagnosed with classic form of CD, but sex did not

significantly affect age at diagnosis (t = 0.87, DF = 91, p = 0.39) nor the duration of symptoms (t = -1.33, DF = 91, p = 0.18).

DISCUSSION

CD is one of the most common diseases worldwide with the prevalence of 1% among Caucasians [8, 9]. Some epidemiological studies showed that the prevalence has increased over past decade, with no significant change in human genome [10]. It points out to the environmental factors and renews their role in the onset of the disease.

In our study, we found that breastfeeding delayed onset of CD in infants. Particularly, breastfeeding during gluten introduction postponed the onset of the disease. According to our study, timing of gluten introduction did not influence the occurrence of CD.

A protective effect of breastfeeding on CD has long been assumed and it occurs through various mechanisms, including presence of numerous nonnutritive factors, like lysozyme lactoferrin, s IgA and others [11, 12]. In addition, breastfeeding is excellent protection from gastrointestinal infections and repeated gastrointestinal infections have been reported to increase the risk of CD [13]. That is why breastfeeding may confer indirect protection from CD [12, 13]. Some studies point to the importance of continuing breastfeeding at the time of gluten introduction [11, 14]. Previous retrospective studies suggested a 'window of opportunity' for primary prevention by introducing gluten between four and six months of age during which breastfeeding provided a protective effect [14, 15, 16]. Small amount of gluten in breast milk helps induce oral tolerance, as is the case with other food allergens [17, 18]. In one recent study, no protective effect of breastfeeding on the development of CD was observed, while in another there was no significant difference in the percentage of children that developed CD among children that were introduced gluten during breastfeeding and in those that were not breastfed at the time of gluten introduction [19, 20, 21].

In our study, the duration of breastfeeding was generally short, and we cannot be positive about its exclusiveness, but it obviously delayed the onset of the disease. Today we know that the type of milk, as well as the mode of the delivery, antibiotics, and stress of any kind, strongly influences gastrointestinal microbiota [22, 23]. We did not investigate the gut microbiota in our participants, but we speculate that breastfeeding can promote and sustain healthy microbiome [24, 25]. This healthy pattern, along with other protective factors in human milk, can promote gluten tolerance, and delay occurrence of the disease, even in patients with strong genetic predisposition [22, 23]. Although we are aware that there are studies that claim breastfeeding at the time of gluten introduction is not protective, in our study we showed that breastfeeding during gluten introduction postponed the disease, which is important, because in our group of patients duration of symptoms for only a few months at the early age was critical for growth, especially for weight gain.

On contrary to old ESPGHAN recommendation that gluten should not be introduced before 17 weeks and not later than at 26 weeks, preferably concurrent with the period of breastfeeding, new ESPGHAN position claims that gluten can be introduced to the infant's diet between the ages of four and 12 months [15, 16, 25]. The age of gluten introduction in infants of this age does not seem to influence the absolute risk of developing CD during childhood. Those recommendations were based on some new prospective, randomized trials [19, 21]. We also did not find the difference in the onset of CD regarding timing of gluten introduction. In our study, only small number of participants consumed gluten prior to the fourth months, and possible in small quantities. Another possible factor, which we did not take into account, was the amount of gluten consumed by the infants.

Additionally, in our study, we found that girls were twice as often affected than boys were which is in accordance to the fact that CD is autoimmune disease, and shares some important features with other autoimmune diseases that are being more prevalent in females than in males [26]. In addition, in our study, sex did not significantly affect the age at diagnosis or the duration of symptoms.

It is well known that genetic predisposition, which all our participants clearly possess, and exposure to gluten are the two most important factors necessary for CD to develop [19, 20, 21]. Early nutrition practices have been studied long and hard, but new studies pointed to other environmental factors to contribute the CD risk [22, 27].

CONCLUSION

In this study, we found that the duration of breastfeeding, and breastfeeding at the time of gluten introduction, postponed the onset of CD in genetically predisposed children up to two years old. We did not confirm the timing of gluten introduction influenced the age of occurrence of CD in this group of children. Our research is a contribution to a very complex nature of CD that requires more investigations not only in the field of genetic predisposition, but also regarding influence of various nutritive and nonnutritive environmental factors on its expression.

Conflict of interest: None declared.

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Утицај дојења и времена увођења глутена на појаву целијачне болести код деце узраста до две године

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САЖЕТАК

Увод/Циљ Класични тип целијачне болести (ЦБ) најчешће се јавља код деце узраста до две године. Циљ ове студије је био да се испита да ли дојење, посебно дојење током увођења глутена, и време увођења глутена утичу на почетак ЦБ у овом добу.

Метод Ретроспективно смо анализирали медицинску документацију 93 деце, 40 у првој и 53 у другој години, са класичном формом ЦБ дијагностикованој у Универзитетској дечјој клиници у Београду између 2000. и 2010. године. Дијагноза ЦБ заснована је на критеријумима Европског удружења за дечју гастроентерологију, хепатологију и нутрицију (*ESPGHAN*) из 1989. године.

Резултати Трајање природне исхране значајно редукује појаву ЦБ у првој години живота ($p = 0,039$, $OR = 1,43$, 95% $CI = 1,019–1,899$). Такође, природна исхрана у време увођења глутена знатно одлаже појаву болести ($F = 1,671$, $t = 2,39$, $p = 0,029$). Време увођења глутена није утицало на узраст појаве ЦБ у овој групи деце.

Закључак Природна исхрана, а посебно природна исхрана у време увођења глутена, одлаже појаву класичне ЦБ код деце узраста до две године. Повезаност појаве ЦБ и времена увођења глутена у овој узрасној групи болесника није доказана.

Кључне речи: класична целијачна болест; деца до две године; дојење; узраст увођења глутена

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Surgical complications of cesarean section

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SUMMARY

Introduction/Objective Cesarean section birth rate has been constantly increasing worldwide over the last decades. The complications of cesarean section that require relaparotomy are rather serious and relatively rare. The aim of this paper is to present the incidence of surgical complications after Cesarean section at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade, Serbia, during a three-year period (2013–2015).

Methods This is a retrospective study. Data obtained from the medical records/histories were used and processed according to descriptive statistical methods.

Results During the observed period, relaparotomy was necessary in 29 (0.44%) women who had a CS. Relaparotomy was performed due to clinically and ultrasonographically evidenced hematoma of the anterior abdominal wall, retroperitoneal hematoma, hemoperitoneum, and development of hemorrhagic shock, complete wound dehiscence or diffuse peritonitis. There were no lethal outcomes after CS followed by these complications at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade.

Conclusion The incidence of relaparotomy in our study is similar to other tertiary institutions, as well as the indications for relaparotomy. While generally observed mortality rate after post-cesarean relaparotomy in developed countries is 2.7%, in our study there were no lethal outcomes.

Keywords: surgical complications; caesarean section; relaparotomy

INTRODUCTION

Cesarean section (CS) birth rate has been constantly increasing worldwide over the last decades [1]. At the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade, this rate has increased over the last years from 30% to 37% in 2015. The increase is attributed to maternal and fetal risk factors, pathological course of pregnancy, and the obstetricians' experience and attitude [2]. More recently, previous delivery by CS frequently imposes the need for every subsequent pregnancy to be delivered in the same way. Maternal morbidity associated with emergency CS is higher compared to elective CS, and maternal complications are more frequent in repeated CS [3].

CS complications requiring relaparotomy are rather serious and relatively rare. The most commonly encountered complications of CS are bleeding and infection [4, 5]. Prolonged labor, longer time period after rupture of the membranes and greater number of vaginal examinations favor postoperative infections, while some risk factors for hemorrhage at CS are uterine atony, *placenta previa*, *placenta accreta*, and the history of previous postpartum hemorrhage [4].

The paper is aimed at presenting incidence of surgical complications after CS at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade, Serbia, during a three-year period (2013–2015).

METHODS

The retrospective case study included patients who underwent relaparotomy during a three-year period (2013–2015) aimed at management of complications associated with CS performed due to relevant indications at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia. Over the aforementioned period, relaparotomy was necessary in 29 patients delivered by CS. Twenty-four women had CS at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, while five patients were transferred to our Clinic after CS was performed at some other maternity hospital in Serbia. Therapeutic relaparotomy was necessary in all of the aforementioned women due to immediate postoperative complications and vital threats.

Indications for relaparotomy, time of onset of complications, intraoperative findings, and the type of reintervention were determined. Postoperative period in the intensive care unit, blood and blood derivative transfusions, choice of antibiotic therapy, total duration of stay in the intensive care unit, total duration of recovery period, and outcome of treatment were followed-up.

The obtained data were processed according to descriptive statistic methods using MS Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS Version 10 (SPSS Inc., Chicago, IL, USA). The investigations were done in accord

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with standards of the institutional committee on ethics. The data were obtained from the patients' medical records/histories. The anonymity of the patients was respected. No intervention was conducted apart from standard clinical procedures, in the best interest of patients.

RESULTS

During the 2013–2015 period, 19,511 deliveries were carried out at the Clinic of Gynecology and Obstetrics. CS was performed in 6,589 women. CS rate was 34%. It was determined that relaparotomy was necessary in 29 patients after CS (0.44%). Out of that number, CS was performed in 24 patients (0.36%) at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, while five patients were transferred to our clinic after CS performed elsewhere in Serbia. In all the women, CS was performed under general anesthesia. Patients who had CS at our Clinic were prophylactically treated with nadroparin (2850 IU) starting 10–12 hours after CS and with antibiotics immediately after the umbilical cord clamping.

The most common indications for CS included previous CS (in most of the patients it was second CS, while in one patient is was fifth CS) and twin pregnancy resulting from *in vitro* fertilization. In 55% of women, emergency CS was performed, while in 45% it was an elective CS.

In 12 women who underwent CS, relaparotomy was performed due to ultrasonographically evidenced hematoma of the anterior abdominal wall. In two of these patients, postoperative course was complicated by the subfebrile condition. Relaparotomy was indicated due to retroperitoneal hematoma in two patients. In four patients urgent relaparotomy was performed due to hemoperitoneum and the development of hemorrhagic shock. Reintervention was necessitated due to complete wound dehiscence in seven patients. In one patient, relaparotomy was required due to the development of diffuse peritonitis, and in one due to the application of Mikulicz tamponade for the correction of hemostasis (Figure 1).

The average time between CS and relaparotomy was 143 hours – i.e. approximately 5.9 days. In the case of wound dehiscence, time to reintervention was approximately 13.2 days, 3.9 days in the case of hematoma. In cases of hemoperitoneum and hemorrhagic shock, the average time before relaparotomy was seven hours, while in the case of diffuse peritonitis it was 158 hours – i.e. approximately 6.5 days.

In all the cases where hematoma was present, its evacuation, complete abdominal cavity exploration, revision of hemostasis, and resuture of the anterior abdominal wall were performed. In two cases, evacuation of hematoma and resuture of the uterus were sufficient for the correction of hemostasis. Ligature of the uterine artery was more frequently needed (in three patients), i.e. ligature of the hypogastric artery (in two patients). Postpartal hysterectomy with adnexal conservation was required in two cases, out of which in one case it was accompanied by Mikulicz tamponade due to iatrogenic injury of the common iliac artery (Table 1).

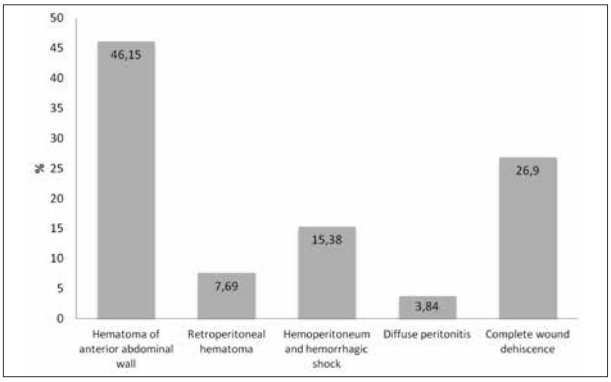

Figure 1. Indications for relaparotomy

Table 1. Type of intervention

Type of intervention	n
Debridement and resuture	9
Evacuation of hematoma, exploration of abdominal cavity, revision of hemostasis and resuture	12 (29)
Suture of the uterus	2
Ligature of the uterine artery	3
Ligature of the hypogastric artery	2
Postpartal hysterectomy	4
Mikulicz tamponade	1

Reintervention was necessary in all five patients admitted to our clinic after CS performed in other institutions. Indications were the following: wound dehiscence (two cases), anterior abdominal wall hematoma (two cases), and diffuse peritonitis associated with the development of sepsis (two cases). Wound dehiscence with or without hematoma was resolved by wound debridement and resuture – evacuation of hematoma that was always accompanied by exploration of the abdominal cavity and revision of the anterior abdominal wall hemostasis. Resuture of the uterus was performed in one case and in two patients who developed peritonitis and sepsis, postpartal hysterectomy with adnexal conservation was mandatory.

In women who underwent relaparotomy due to hematoperitoneum or retroperitoneal hematoma, the intraoperative blood salvage / cell saver procedure was followed. This method is associated with fewer adverse effects compared to allogeneic blood transfusion. Autologous salvaged blood provides better quality red blood cells that have not been subjected to the detrimental effects of blood storage.

In all the patients with massive hemorrhage, the following procedures were conducted:

– preservation of intravascular volume, either by intraoperative blood salvage, or by using plasma expanders;
– use of antifibrinolytics (tranexamic acid);
– use of tissue adhesives and fibrin glues;
– administration of desmopressin;
– if necessary, inotropic drugs.

Perioperatively, as a relevant method of assessing coagulation, rotational thromboelastometry was used.

In the course of reintervention, the patients received 875 mL of blood and 425 mL of plasma on average, as

well as 4.6 doses of cryoprecipitate on average. During the immediate postoperative course, all the patients were on intensive care units, with their stay averagely lasting 3.2 days, and received over the period additional 405 mL of blood and 315 mL of plasma on average as well as four average doses of cryoprecipitates. They were most commonly treated with triple antibiotic therapy. All the patients responded well to the applied measures and all were discharged to outpatient treatment in good general condition. There were no lethal outcomes.

DISCUSSION

Based on the literature data, relaparotomy rate after CS ranges 0.2–0.9% [4, 6, 7, 8]. In our study, relaparotomy was indicated in 0.44% of the patients, which falls within the range observed in other countries. The difference in relaparotomy rates in different settings may be explained by conditions offered by the medical institutions of higher level, possibilities of appropriate diagnostic measures and monitoring in intensive care units, technical and staff potentials and experience related to the treatment of these patients. As a rule, the rate is lower in tertiary level institutions [5].

Hemodynamic instability as a consequence of suspected intraabdominal and/or vaginal bleeding is reported to be the most common indication for relaparotomy after CS, accounting for approximately 66–68% of the cases [9, 10, 11]. For these reasons, relaparotomy is most commonly performed within the first five hours of CS, which corresponds to clinical picture of hemodynamic instability [6]. In our study, hemoperitoneum and hemorrhagic shock were not so common. They were recorded in 15.38% of all surgically corrected complications of CS, and they were resolved within 10 hours of CS. Somewhat longer period of approximately two weeks before treatment is reported in cases of infected hematomas. In all our studied patients with wound dehiscence, time to reintervention was approximately 13 days, while in cases with hematomas it was approximately four days.

In a large study that included 28,799 patients, relaparotomy was performed after CS in 35 patients for the following indications: intraabdominal bleeding (34.2%), intraabdominal hematoma (22.8%), and atony (8.6%) [11]. In a study by Ragab et al. [7], the most common indication for post-cesarean relaparotomy was internal hemorrhage (hemoperitoneum) (66.6%), while maternal mortality occurred in 16.6% of the patients. Also, in a study by Huras et al. [8], hematoperitoneum was the main indication for post-CS relaparotomy. On the other hand, the most predominant indications in our study were hematoma (46.15%) and wound dehiscence (26.92%), followed by hemorrhagic shock (15.38%) and diffuse peritonitis (3.84%), and there were no lethal outcomes. Evidenced risk factors in a study by Gedikbasi et al. [12] included three and more previous CS, placental abruption, and multifetal pregnancies, which is consistent with our findings. If hysterectomy is necessary after CS, it is most commonly the result of uterine atony accompanied by severe bleeding with *placenta accreta* also being a significant risk factor [13].

In women who underwent relaparotomy due to hematoperitoneum or retroperitoneal hematoma, the intraoperative blood salvage / cell saver procedure was conducted. This is now standard procedure even for routine cesarean delivery in tertiary centers [14]. However, current guidelines do not support the routine use of cell salvage during caesarean section, but its use is considered rational in women at high risk of hemorrhage or if unanticipated bleeding develops during CS [15].

Assumption that the duration of CS may be associated with the higher risk of relaparotomy was not confirmed in our study [5]. The incidence of maternal mortality after CS in developed countries (USA) is 13.3 per 100,000 inhabitants, while in vaginal delivery the incidence is 3.6 per 100,000. General incidence of severe complications associated with CS is 9.2%, with total maternal mortality being 2.7% [6, 16, 17, 18]. During the three-year period (2013–2015) there were no lethal outcomes after CS at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade.

CONCLUSION

The incidence of relaparotomy in our study is similar to other tertiary institutions, as well as the indications for relaparotomy. While generally observed mortality rate after post-cesarean relaparotomy in developed countries is 2.7%, there were no lethal outcomes in our study.

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Conflict of interest: None declared.

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Хируршке компликације царског реза

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САЖЕТАК

Увод/Циљ Број порођаја путем царског реза (ЦР) стално се повећава широм света током последњих деценија. Компликације царског реза које захтевају релапаротомију релативно су ретке али прилично озбиљне. Циљ овог рада је да се покажу инциденције хируршких компликација након ЦР на Клиници за гинекологију и акушерство Клиничког центра Србије у Београду током трогодишњег периода (2013–2015).

Методе Студија је дизајнирана по ретроспективном типу. Коришћени подаци добијени су из медицинске документације и обрађени према дескриптивним статистичким методама.

Резултати Током посматраног периода релапаротомија је била неопходна код 29 (0,44%) жена код којих је рађен царски рез. Релапаротомија је извршена због клинички и ултрасонографски доказаног хематома предњег трбушног зида, ретроперитонеалног хематома, интраабдоминалног крварења и развоја хеморагичног шока, потпуне дехисценције ране или дифузног перитонитиса. На Клиници за гинекологију и акушерство Клиничког центра Србије у Београду у проучаваном периоду није било смртних исхода услед компликација ЦР.

Закључак Инциденција релапаротомије у овој студији слична је као у другим терцијарним установама, као и индикације за релапаротомију. Генерално гледајући, стопа морталитета после релапаротомије због компликација ЦР у развијеним земљама износи 2,7%, док у овој студији није било смртоносних исхода.

Кључне речи: хируршке компликације; царски рез; релапаротомија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Differences in endometrial carcinoma presentations and characteristics in pre- and postmenopausal women

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SUMMARY

Introduction/Objective As of recently, an increasing number of premenopausal women is being diagnosed with endometrial carcinomas.
The objective of our study was to determine if routinely collected clinical and imaging parameters, implying on tumor characteristics, are different in pre- and postmenopausal endometrial carcinoma patients, enabling their appropriate preoperative evaluation.

Methods The study included all patients (n = 209) operated on due to endometrial carcinoma over a period of three years. The diagnosis was based on histopathological findings of exploratory curettage. Medical history was taken for all the patients and they were divided regarding menopausal status. On preoperative ultrasound scan, the endometrial echo pattern was established. The existence of myomas, adnexal masses, free fluid in the abdomen or uterine cavity was noted. Magnetic resonance imaging detected the presence of pelvic metastases and tumor spreading into the uterine cavity, myometrium, cervix, and lymph nodes. Postoperatively, histopathological findings, the tumor stage and grade were established.

Results The majority of women were postmenopausal and secundiparous. Significantly more patients were obese, especially the postmenopausal ones (p = 0.001). Most tumors were endometrioid adenocarcinomas regardless of menopausal status. Irregular/abnormal bleeding (p = 0.037), presence of ascites (p = 0.010), obesity (p = 0.046), and lower parity (p = 0.016) correlated with postmenopausal status. Large exophytic endometrial carcinomas were predominant in younger patients (p = 0.026). Endometrial carcinomas were significantly more often diagnosed in the II FIGO stage in premenopausal patients. There were no other significant differences (endometrial thickness, uterine homogeneity, echogenicity, tumor infiltration and spreading, histopathological type and grade) between pre- and postmenopausal endometrial carcinoma patients.

Conclusions Few differences between pre- and postmenopausal endometrial carcinoma patients existed and the most prominent ones were obesity, parity, irregular/abnormal bleeding, and tumor growth into the cavity.

Keywords: endometrial carcinoma; menopausal status; BMI; irregular abnormal bleeding; preoperative evaluation; ultrasound scan and MRI

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INTRODUCTION

Endometrial carcinoma is one of the most common malignant tumors of the female genital system. It accounts for about 4% of all malignancies in women worldwide [1]. Typical symptoms are irregular/abnormal bleeding and pelvic pain [2]. So far established risk factors, such as obesity, impaired lipid and carbohydrate metabolism, infertility and low parity, late onset of menopause and anovulatory cycles, are related to hyperestrogenism [3, 4].

Occurrence of endometrial carcinoma increases with age and it usually arises in postmenopausal women. However, as of recently, an increasing number of younger premenopausal women have been diagnosed with endometrial carcinoma, possibly due to an epidemic of obesity and physical inactivity even of young girls [1, 5].

The differential diagnosis of different pathological conditions on the base of secretory endometrium can be difficult [5]. Good preoperative discrimination between benignant and malignant endometrial proliferations is essential for appropriate therapeutic approach. In postmenopausal women, vaginal bleeding and/or endometrial thickness measured by transvaginal ultrasonography above 5 mm are considered to be very suspicious of endometrial carcinoma and present an indication for exploratory curettage [6]. However, currently

there are no algorithms based on clinical, laboratory, and imaging parameters for the assessment of premenopausal women that may have endometrial carcinoma.

Moreover, ultrasonographic examination sometimes has low diagnostic reliability with numerous false positive findings [7]. Although the exact diagnosis is achieved by examination of endometrial tissue samples obtained on fractionated exploratory curettage or hysteroscopy with endometrial biopsy, detection of malignant invasion of the myometrium is usually diagnosed by histopathological analysis only after hysterectomy [8].

The objective of the study was to determine if routinely collected clinical and imaging parameters, implying on tumor characteristics [histopathological findings (HP), grade, stage], are different in pre- and postmenopausal women, enabling appropriate preoperative evaluation of different age group patients with endometrial carcinoma.

METHODS

The study included all patients who were operated at the Clinic of Obstetrics and Gynecology, Clinical Center of Serbia, over a three-year period (January 1, 2011 to December 31, 2013) due to endometrial carcinoma. The initial diagnosis and the decision for operative treatment were based on HP of exploratory curettage that all patients had either due to irregular/abnormal bleeding or endometrial thickening registered by ultrasound on a regular gynecological check-up. Upon admission for operation, the standard medical history [irregular/abnormal bleeding, age, parity, menopausal status and the age of menopause, the use of hormone replacement therapy or tamoxifen, comorbidities like breast carcinoma, hypertension, diabetes mellitus, etc.] was taken for all patients and their body mass index (BMI) was calculated. Preoperatively, all the patients had a detailed trans-vaginal ultrasound (TVUS) scan with the measurement of endometrial thickness. The homogeneity and echogenicity of the uterine tissue were evaluated. The existence of myomas and adnexal masses were noted. The presence of free fluid in the abdomen (ascites) as well as in the uterine cavity was also registered. Furthermore, the patients underwent the magnetic resonance imaging (MRI) of the pelvis. By analyzing the MRI images, we determined tumor spreading into the uterine cavity, the myometrium, the cervix, and the lymph nodes, as well as the presence of pelvic metastases. Postoperatively, HP of the tumor (type, grade, and stage) were analyzed. Staging was performed according to the new International Federation of Gynecologist and Obstetricians (FIGO) classification. All the women were divided regarding their menopausal status and the obtained data was analyzed accordingly.

Upon admission to hospital, all the women gave informed consent to all the diagnostic and therapeutic procedures required for this study, as well as to their enrolment in the study sample. Study procedure was performed in accordance with the ethical standards and it was approved by the Clinic Ethics Committee.

For the statistical analysis, we used methods of descriptive and analytical statistics (percentages, χ^2 test, Kruskal–Wallis nonparametric ANOVA, Spearman correlation, binary linear regression) and IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The study involved 209 women who were operated on due to endometrial carcinoma at the Clinic for Obstetrics and Gynecology, Clinical Center of Serbia, throughout a period of three years (2011–2013).

In the examined population we registered significantly more postmenopausal (168; 80.4%) than premenopausal (41; 19.6%) women with endometrial carcinomas ($\chi^2 = 77.172$; $p = 0.001$). The majority of patients (n = 83; 39.71%) were in their 60s, with an average age of 63.41 ± 9.92 years (min. = 36; max. = 85). The mean BMI of investigated patients was 28.70 ± 5.42 (min. = 17; max. = 40.9; premenopausal BMI 28.97 ± 6.66; postmenopausal BMI 28.63 ± 5.1). Significantly more women were obese (BMI > 25 kg/m^2), especially in the postmenopausal group. The majority of women of both groups had two births and no abortions (Table 1).

Significantly more patients neither had previously diagnosed breast carcinoma nor administered therapy for it. Only nine women had ever used hormonal replacement therapy. Investigated patients mostly did not have other comorbidities, but hypertension was very frequent. The majority of patients had irregular/abnormal bleeding. This was especially prominent for postmenopausal women (Table 1).

The mean endometrial thickness on preoperative TVUS scan was 12.21 ± 8.46 mm (min. = 3 mm; max. = 39 mm; premenopausal 13.45 ± 10.8; postmenopausal 11.91 ± 7.79). Significantly more women had pathologically increased endometrial thickness regardless of their menopausal status (Table 2). Most tumors had exophytic growth filling the uterine cavity. On TVUS scan, significantly more both pre- and postmenopausal women had homogenous but hyperechogenic endometrial presentation (Table 2). Significantly more women, regardless of their menopausal status, neither had TVUS findings of fluid in the uterus or abdomen, nor pelvic metastases, myomas or adnexal tumors on MRI. Myometrium was usually infiltrated less than one-third of its thickness in both investigated groups (Table 2).

Four different histopathological diagnoses of endometrial carcinomas were registered. The majority of tumors were endometrioid adenocarcinomas regardless of menopausal status. Nevertheless, there were no cases of carcinosarcoma and clear cell carcinomas in the premenopausal group. The majority of carcinomas in postmenopausal women were in FIGO stage I with grade G1, NG1, while in the premenopausal group endometrial carcinomas were mostly registered in stage II and their predominant grade was G2, NG2 (Table 3).

Having irregular/abnormal bleeding, obesity and lower parity were significantly positively correlated with postmenopausal status. Ascites registered by MRI was

Table 1. Frequency of assessed characteristics in pre- and postmenopausal women and the significance of differences between tested parameter categories in the group

Parameter	Category	Premenopausal women		Postmenopausal women		Total population	
		Number	%	Number	%	Number	%
Body mass index	appropriate	15	36.6	37	22	52	24.9
	obesity	26	63.4	131	78	157	75.1
	p	0.086		0.001		0.001	
Irregular/abnormal bleeding	no	14	34.1	32	19	46	22
	yes	27	65.9	136	81	163	78
	p	0.042		0.001		0.001	
Parity	0	6	14.6	29	17.3	35	16.8
	1	15	36.6	40	23.8	55	26.3
	2	13	31.7	87	51.8	100	47.8
	3 and more	7	17.1	12	7.1	19	9.1
	p	0.125		0.001		0.001	
Breast carcinoma	no	38	92.7	157	93.5	195	93.3
	yes	3	7.3	11	6.5	14	6.7
	p	0.001		0.001		0.001	
Tamoxifen use	no	40	97.6	157	93.5	197	94.3
	yes	1	2.4	11	6.5	12	5.7
	p	0.001		0.001		0.001	
Hormonal substitution	no	36	87.8	164	97.6	200	95.7
	yes	5	12.2	4	2.4	9	4.3
	p	0.001		0.001		0.001	
Comorbidities	no	25	61	59	35.1	84	40.2
	HT	8	19.5	73	43.5	81	38.8
	diabetes mellitus	1	2.4	6	3.6	7	3.3
	both HT / DM or other	7	17.1	30	17.9	37	17.7
	p	0.001		0.001		0.001	

p – difference between categories of tested parameter in the group; HT – hypertension; DM – diabetes mellitus

Table 2. Examined parameters of tumors in pre- and postmenopausal women and the significance of differences between tested parameter categories in the group

Parameter	Category	Premenopausal women		Postmenopausal women		Total population	
		Number	%	Number	%	Number	%
Homogeneity on TVUS	no	13	31.7	75	44.6	88	42.1
	yes	28	68.3	93	55.4	121	57.9
	p	0.019		0.165		0.001	
Echogenicity on TVUS	normal	16	39	74	44	90	43.1
	hyper	25	61	94	56	119	56.9
	p	0.160		0.123		0.001	
Endometrium on TVUS	≤ 5 mm	9	22	36	21.4	45	21.5
	> 5 mm	32	78	132	78.6	164	78.5
	p	0.001		0.001		0.001	
IU fluid on TVUS	no	29	70.7	137	81.5	166	79.4
	yes	12	29.3	31	18.5	43	20.6
	p	0.008		0.001		0.001	
Myoma on TVUS	no	31	75.6	129	76.8	160	76.6
	yes	10	24.4	39	23.2	49	23.4
	p	0.001		0.001		0.001	
Adnexal tumor on TVUS	no	36	87.8	150	89.3	186	89
	yes	5	12.2	18	10.7	23	11
	p	0.001		0.001		0.001	
Ascites on TVUS	no	33	80.5	157	93.5	190	90.9
	yes	8	19.5	11	6.5	19	9.1
	p	0.001		0.001		0.001	
Uterine cavity MRI	without tumor	10	24.4	73	43.5	83	39.7
	filled with tumor	31	75.6	95	56.5	126	60.3
	p	0.001		0.001		0.001	

Parameter	Category	Premenopausal women		Postmenopausal women		Total population	
		Number	%	Number	%	Number	%
Cervix on MRI	benignant	28	68.3	115	68.5	143	68.4
	malignant cells	13	31.7	53	31.5	66	31.6
	p	0.019		0.001		0.001	
Lymph nodes on MRI	benignant	35	85.4	150	89.3	185	88.5
	malignant cells	6	14.6	18	10.7	24	11.5
	p	0.001		0.001		0.001	
MRI pelvic metastases	no	35	85.4	155	92.3	190	90.9
	yes	6	14.6	13	7.7	19	9.1
	p	0.001		0.001		0.001	
Myometrium infiltration on MRI	unaffected	4	9.8	13	7.7	17	8.1
	< 1/3	23	56.1	105	62.5	128	61.2
	1/3–2/3	13	31.7	45	26.8	58	27.8
	whole	1	2.4	5	3	6	2.9
	p	0.001		0.001		0.001	

TVUS – transvaginal ultrasound; MRI – magnetic resonance imaging; IU – intrauterine

Table 3. Postoperative diagnoses of tumors in pre- and postmenopausal women and the significance of differences between tested parameter categories in the group

Parameter	Category	Premenopausal women		Postmenopausal women		Total population	
		Number	%	Number	%	Number	%
Tumor grade	G1, NG1	17	41.5	75	44.6	92	44
	G1, NG2	21	51.2	63	37.5	84	40.2
	G2, NG1	2	4.9	17	10.1	19	9.1
	G2, NG2	1	2.4	10	6	11	5.3
	G2, NG3	0	0	3	1.8	3	1.4
	p	0.001		0.001		0.001	
FIGO stage	Ia	11	26.8	38	22.6	49	23.4
	Ib	4	9.8	36	21.4	40	19.1
	Ic	1	2.4	21	12.5	22	10.5
	IIa	14	34.1	30	17.9	44	21.1
	IIb	7	17.1	19	11.3	26	12.4
	IIIa	1	2.4	14	8.3	15	7.2
	IIIb	2	4.9	3	1.8	5	2.4
	IIIc	1	2.4	7	4.2	8	3.8
	p	0.001		0.001		0.001	
HP DG	Endometrioid adenocarcinoma	39	95.1	150	89.3	189	90.4
	Carcinosarcoma	0	0	5	3	5	2.4
	Adenosquamous carcinoma	2	4.9	8	4.8	10	4.8
	Clear cell carcinoma	0	0	5	3	5	2.4
	p	0.001		0.001		0.001	

HP DG – histopathological diagnosis; p – difference between categories of tested parameter in the group

significantly more frequent in postmenopausal women, while exophytic growth of endometrial carcinoma that filled out the uterine cavity was usually seen in younger patients. Moreover, FIGO stage was more advanced in premenopausal women. There were no other significant correlations or differences in examined parameters between pre- and postmenopausal endometrial carcinoma patients (Table 4).

A significant equation was constructed that shows which clinical, TVUS, and MRI parameters assessed together can be used for differentiation between pre- and postmenopausal women with endometrial carcinoma (B = 1.410; Wald = 65.558; Exp (B) = 4.098; R^2 Nagelkerke = 0.252; total classification = 67.3%; χ^2 = 36.015; p = 0.011). According to the obtained model, the strongest differences are in irregular/abnormal bleeding (p = 0.023), parity (p = 0.004), and tumor growth into the cavity (p = 0.035).

DISCUSSION

Endometrial carcinoma is usually registered in women older than 60 years [9]. This finding is consistent with our study, in which the average age of patients was 63 years. Still, the youngest patient was only 36 years old.

In some reports, older age, tumor grade, involvement of the lower uterine segment and lymphovascular infiltration were proven as significant predictive factors of endometrial malignancy, influencing also the patient's survival [9].

Table 4. Correlations and differences between investigated parameters regarding the menopausal status (pre- and postmenopausal) of women with endometrial carcinoma

Parameters	Correlations		Differences	
	Spearman ρ	p	KW χ^2	p
Body mass index	0.111	0.046	0.026	0.871
Irregular/abnormal bleeding	0.145	0.037	4.356	0.037
Breast carcinoma	-0.012	0.861	0.031	0.860
Tamoxifen use	0.070	0.313	1.023	0.312
Comorbidities	0.158	0.023	5.165	0.023
Parity	-0.166	0.016	5.730	0.017
Endometrium mm TVUS	-0.026	0.704	0.145	0.703
Homogeneity on TVUS	-0.104	0.134	2.251	0.133
Echogenicity on TVUS	-0.040	0.563	0.338	0.561
IU fluid on TVUS	-0.106	0.126	2.348	0.125
Myoma on TVUS	-0.011	0.874	0.025	0.874
Adnexal tumor on TVUS	-0.019	0.787	0.073	0.786
Pelvic metastases	-0.095	0.170	1.887	0.170
Ascites on TVUS	0.179	0.009	6.671	0.010
Growth in uterine cavity on MRI	-0.155	0.025	4.978	0.026
Cervix spreading on MRI	-0.001	0.984	0.000	0.984
Lymph nodes on MRI	-0.049	0.483	0.496	0.481
Myometrium on MRI	-0.019	0.789	0.072	0.789
Tumor grade	0.025	0.715	0.134	0.715
FIGO stages (I, II, III)	-0.038	0.581	6.607	0.037
HP DG	0.079	0.258	1.287	0.257

HP DG – histopathological diagnosis; TVUS – transvaginal ultrasound; MRI – magnetic resonance imaging; IU – intrauterine; HRT – hormone replacement therapy; KW – Kruskal–Wallis

Studies also confirmed that myometrial invasion, tumor diameter, cervical stromal invasion, and lymphovascular space invasion were the most important parameters for preoperative evaluation and therapy type determination in patients with endometrial malignancies [10].

According to the literature data, only a few studies have evaluated the influence of risk factors for endometrial carcinoma in women of different ages (younger – premenopausal, and older – postmenopausal). Early menarche and nulliparity were correlated with increased endometrial carcinoma risk in premenopausal, but not in postmenopausal women in some investigations. Late menopause showed a stronger association with endometrial carcinoma in older (over 65 years) than in premenopausal patients [11, 12]. Parity in the population we tested was found to be a significant predictive factor for malignancy only in postmenopausal women.

Women with elevated endogenous estrogen levels have an increased risk of endometrial carcinoma. The diagnosis of polycystic ovarian syndrome has been made in up to 30% of cases with endometrial carcinoma in selected groups of premenopausal women [12]. Obesity was confirmed through numerous studies as the main risk factor for endometrial carcinoma [11, 12]. Some authors suggest that this association is consistent only for postmenopausal women, while others confirmed correlation in premenopausal women as well [12]. In obesity, there is an increased level of free estrogen due to increased conversion of fatty tissues from androstenedione. Estrogen leads to chronic proliferation of endometrial cells, increasing the risk of carcinoma occurrence [13, 14]. In our population, 75% of the patients were obese (BMI > 25), indicating that being overweight raises the risk of endometrial malignancy. Still, the obesity was proven as endometrial carcinoma risk factor only for postmenopausal patients.

Although the histopathological assessment of the endometrial biopsy remains the gold standard, TVUS is considered to be the first step in any woman presenting with abnormal uterine bleeding [15]. A thin and regular endometrial line clearly visualized throughout the uterus is associated with a very low risk of endometrial carcinoma. Some authors believe that if endometrium is thinner than 5 mm measured by TVUS even with postmenopausal bleeding, the risk for carcinoma is low but not ruled out, especially in cases of persistent bleeding [16]. The value of TVUS in symptomatic premenopausal women and those using hormone substitution therapy is lower because the endometrial thickness regularly varies with changes of hormones during cycle. The data we obtained confirm previous findings which have shown that endometrial thickness above 5 mm in women with postmenopausal bleeding can be considered an accurate diagnostic parameter of endometrial malignancy [6]. However, there were no significant differences in endometrial thickness of pre- and postmenopausal women with endometrial carcinoma.

MRI is an important imaging modality in the preoperative assessment of endometrial carcinoma patients providing valuable data regarding lesion location and qualitative information for preoperative staging [17, 18]. MRI is able to accurately predict (sensitivity and specificity above 85%) cervical involvement in endometrial carcinoma patients and allows an adequate treatment decision [17, 19]. Some authors believe that reliability of MRI is better in the postmenopausal than in premenopausal women [18]. MRI findings in our population were mostly similar (echogenicity, homogeneity, endometrial thickness, level of cervical and myometrial invasion at the time of diagnosis, etc.) between pre- and postmenopausal women.

According to some available literature data, tumor volume influences the rate of endometrial carcinoma progression [20]. Based on our results, the size of tumor within the uterine cavity measured by MRI (filling the whole cavity) was confirmed to be a relevant parameter in premenopausal patients.

Some researchers have shown that positive peritoneal fluid cytology represents a marker for shorter time to recurrence of the disease and decreased survival rate of patients with endometrial carcinoma [21]. In the study population, only a few patients had ascites and enlargement of lymph nodes. Nevertheless, free fluid in the abdominal cavity seen by MRI was registered more often in the postmenopausal women.

Even though in the overall tested population the majority of carcinomas were diagnosed in early stages, numerous carcinomas in premenopausal patients were at FIGO stage II at the time of diagnosis. This might be due to the fact that irregular/abnormal bleeding in younger women is occasionally misdiagnosed and inappropriately treated.

CONCLUSION

We confirmed that endometrial carcinoma is significantly more frequent in postmenopausal than in premenopausal women, while endometrioid adenocarcinoma is the most frequent histopathological diagnosis regardless of the menopausal status. There are few differences in endometrial carcinoma presentation/characteristics between pre- and postmenopausal patients. The presence of ascites was more frequent in postmenopausal women, while large exophytic endometrial carcinomas were predominant in younger patients. Endometrial thickness, uterine homogeneity, echogenicity, tumor infiltration and spreading at the time of diagnosis were similar between pre- and postmenopausal women with endometrial carcinomas. Although most women had BMI > 25, obesity presents an endometrial carcinoma risk factor for postmenopausal patients. Postmenopausal bleeding enables a diagnosis at earlier stages than irregular/abnormal bleeding in premenopausal patients. Ultrasound and MRI are appropriate diagnostic tools in patients with endometrial carcinoma, but their findings are not reliable for predicting tumor stage, grade, or exact histopathological diagnosis.

Conflict of interest: None declared.

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Разлике у презентацији и карактеристикама карцинома ендометријума код жена у пременопаузи и постменопаузи

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САЖЕТАК

Увод/Циљ У последње време карцином ендометријума се дијагностикује код све већег броја жена у пременопаузи. Циљ студије је био да се утврди да ли су рутински прикупљени клинички и *imaging* параметри, који могу да укажу на карактеристике тумора, различити код болесница са карциномом ендометријума у пременопаузи и постменопаузи, што би омогућило њихову правилну преоперативну процену.

Методе Студија је обухватила све болеснице (n = 209) оперисане због карцинома ендометријума током три године. Дијагноза је заснована на хистопатолошким налазима експлоративне киретаже. За све болеснице узета је детаљна анамнеза и оне су подељене према свом менопаузном статусу. На преоперативном ултразвучном прегледу одређен је „ехо образац" ендометријума. Регистровано је постојање миома, аднексалних маса, слободне течности у абдомену или у кавуму утеруса. Магнетном резонанцом детектовано је присуство метастаза у малој карлици и ширење тумора на кавум утеруса, миометријум, грлић и лимфне чворове. Постоперативно су одређени хистопатолошки тип, стадијум и градус тумора.

Резултати Већина болесница су биле у постменопаузи и секундипаре. Значајно више болесница су биле гојазне, наврочито у постменопаузи (p = 0,001). Већина тумора су били ендометриоидни аденокарциноми без обзира на менопаузни статус. Нередовно/абнормално крварење (p = 0,037), присуство асцитеса (p = 0,010), гојазност (p = 0,046) и нижи паритет (p = 0,016) били су повезани са постменопаузним статусом. Велики егзофитични карциноми ендометријума били су доминантан налаз код млађих болесница (p = 0,026). Карцином ендометријума је значајно чешће дијагностикован у *FIGO* стадијуму II код болесница у пременопаузи. Није било других значајних разлика (дебљина ендометријума, хомогеност и ехогеност материце, туморска инфилтрација и ширење, хистопатолошки тип и градус) између болесница са карциномом ендметријума у пременопаузи и постменопаузи.

Закључак Мале разлике су постојале између болесница са ендометријалним карциномом у пременопаузи и постменопаузи, а најзначајније су биле гојазност, паритет, нередовно/абнормално крварење и раст тумора ка материчној шупљини.

Кључне речи: карцином ендометријума; менопаузни статус; ИТМ; ирегуларно крварење; преоперативна процена; ултразвучни преглед и магнетна резонанца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of remifentanil used during caesarean section on maternal hemodynamics and neonatal outcome – comparison of two dosing regimens

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SUMMARY

Introduction/Objective To present and compare maternal and neonatal effects of two remifentanil dosing regimens, used during induction-delivery period of elective caesarean section in attempt to attenuate maternal cardiovascular response to surgical stress.

Methods Seventy-seven ASA I-II parturients were randomly divided into three groups and received the following: A – 1 μg/kg remifentanil immediately before the induction to anesthesia followed by 0.15 μg/kg/min infusion, interrupted after skin incision; B – 1 μg/kg remifentanil bolus immediately before the induction; C – no remifentanil until delivery. Hemodynamic (blood pressure, heart rate) and bispectral index changes after endotracheal intubation, skin incision, peritoneal incision and delivery, intraoperative anesthetics consumption and neonatal outcome have been compared between the groups.

Results Hemodynamic response to intubation was significantly attenuated ($p < 0.001$) in groups A and B compared to C. Hemodynamic response to skin incision, peritoneal incision and delivery was significantly attenuated in group A compared to B and C. Thiopentone dose in groups A and B was lower than in group C ($p < 0.001$); sevoflurane and remifentanil consumption was less in group A compared to B and C ($p < 0.001$). Apgar scores at 1st minute were ≥ 8 in all neonates, with no differences in neonatal heart rate, oxygen saturation and umbilical blood gas values (all within normal range).

Conclusion 1 μg/kg remifentanil bolus followed by 0.15 μg/kg/min stopped after skin incision, successfully blunted maternal hemodynamic stress response throughout whole induction-delivery period, reduced anesthetic consumption, without affecting neonatal outcome, so it can be considered effective as well as safe to use during induction-delivery period of caesarean section.

Keywords: anesthesia; obstetrical; remifentanil

INTRODUCTION

When performing general anesthesia for caesarean delivery, anesthetists can experience conflicting situation called "the dilemma of obstetrics anesthesia." It is important to ensure an appropriate maternal level of anesthesia, while avoiding neonatal respiratory depression caused by the medications that parturient receives [1, 2]. Resolving the problem of neonatal well-being by reducing as much as possible doses of anesthetics given to the mother and omission of opioids during induction to delivery period (I-D), result in light anesthesia with increased risk of intraoperative awareness and exaggerated neuroendocrine stress response to surgical stress, possibly leading to severe cardio- and cerebrovascular complications [1–5]. Remifentanil, ultra-short acting synthetic opioid, could be the appropriate drug to use for the attenuation of maternal stress response during the I-D interval, where a brief but intense analgesia without prolonged effect is desirable [2, 4, 6–9]. Remifentanil has rapid onset of action (1–1.5 min.), rapid redistribution and metabolism dependent on nonspecific tissue and plasma esterases; its context sensitive half time is three min; it crosses the placenta, but appears to be rapidly metabolized and redistributed in the fetus, leaving the small possibility of neonatal adverse effects [2, 4, 7, 9, 10].

In studies reporting the use of remifentanil during the I-D period dosing regimens were different; hemodynamic stability was often achieved at the expense of neonatal respiratory depression [2, 4, 6–9, 11–16]. In the present study, we investigated the effects of two remifentanil dosing regimens, used during the I-D period, on maternal hemodynamics and neonatal outcome in attempt to find the best compromise between the attenuation of maternal stress response and avoidance of neonatal adverse effects.

METHODS

The study was institutionally approved and has Medical faculty of Niš Research Ethics Committee approval No 12-2466-1. Seventy-seven ASA physical status I-II women with singleton term pregnancy, who were scheduled for elective caesarean section and have given written informed consent, were enrolled in this

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prospective, randomized controlled study, performed at Clinic of Gynecology and Obstetrics Niš, from April 2015 until July 2017. Exclusion criteria were maternal morbidity and signs of fetal compromise. All patients refused or had absolute/relative medical contraindications to regional anesthesia.

In the operating room, patients were placed supine with left uterine displacement, standard monitoring – non-invasive blood pressure, electrocardiography, pulse oxymetry, capnography (using bed side monitor, model BSM-2301k, Nihon Kohden Corporation, Tokyo, Japan) and bispectral index (BIS) electroencephalogram (BIS-Vista monitoring system Norwood, Massachusetts, USA) was initiated and two intravenous lines established, one for remifentanil infusion (using Perfusor fm B/Brown, Melsungen AG, Germany), the other for the administration of other medications and fluids.

Patients were randomly allocated (using envelope method) to one of the following groups:

1. A – 31 patients received 1 μg/kg remifentanil bolus over 30 seconds immediately before the induction, followed by 0.15 μg/kg/min infusion that was stopped after the skin incision.
2. B – 27 patients received 1 μg/kg remifentanil bolus over 30 seconds immediately before the induction
3. C (control) – 19 patients did not receive remifentanil until delivery of the baby.

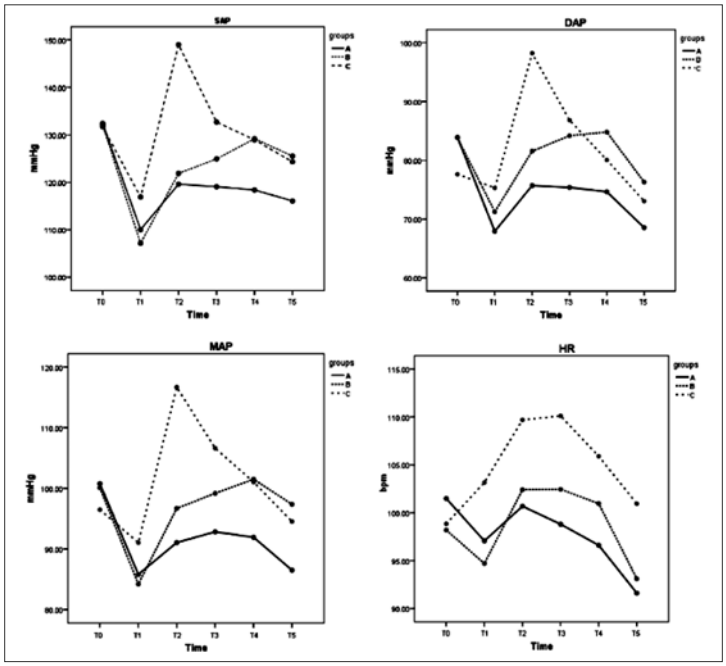

Figure 1. Serial systolic, diastolic, main arterial pressure and heart rate measurements – from T0 to T5; SAP – systolic arterial pressure; DAP – diastolic arterial pressure; MAP – main arterial pressure; HR – heart rate; T0 – basal values; T1 – induction of anesthesia; T2 – intubation; T3 – skin incision; T4 – peritoneal incision; T5 – delivery; group A – remifentanil bolus + infusion; group B – remifentanil bolus; group C – control

Anesthesia was induced with thiopentone, starting with 3 mg/kg, followed by additional 25 mg boluses until adequate depth of anesthesia had been reached (BIS values under 60, but not below 40); succinylcholine was administered in a dose of 1.5 mg/kg. Anesthesia was maintained with 1–1.5% end-tidal sevoflurane and 50% nitrous oxide in oxygen. Further muscle relaxation has been provided with rocuronium 0.6 mg/kg. The lungs were mechanically ventilated to maintain end-tidal PCO_2 of 28–32 mmHg, with fresh gas flow of 6 l/min.

SAP, DAP, MAP (systolic, diastolic, main arterial pressure, respectively), HR (heart rate) and BIS were measured and recorded at basal time (T0) and 30 seconds after induction to anesthesia (T1), endotracheal intubation (T2), skin incision (T3), peritoneal incision (T4), delivery (T5) and also in two-minute intervals from the delivery until the end of operation.

After delivery, neonatologist blinded to group assignment assessed neonates and recorded the time to sustained respiration, Apgar score at 1st and 5th minute, HR, SpO_2 and, if required, resuscitative measures (tactile stimulation, beg-mask ventilation, endotracheal intubation or naloxone administration). We took arterial and venous blood samples (in heparinized syringes) from a double-clamped umbilical cord, for blood gas analysis (using Gem Premier 3000 Blood Gas/ Electrolyte Analyzer, Model 5700, Instrumentation Laboratory Company, Bedford, Massachusetts, USA).

In the later course of the operation, sevoflurane and remifentanil were titrated according to BIS values and presence/absence of signs of intraoperative surgical stress (autonomic, somatic, hemodynamic). Sevoflurane and nitrous oxide were discontinued at the moment of skin closure, residual neuromuscular block antagonized using neostigmine and atropine, and remifentanil infusion rate reduced to 0.07 μg/kg/min. The trachea was extubated when spontaneous respiratory rate reached > 10 breaths/min., end-tidal CO_2 < 45 mmHg and the patient became responsive to verbal commands. Remifentanil infusion was then stopped. The presence of intraoperative awareness was checked two and 24 hours after the operation by using Brisce questionnaire: What is the last thing you remember before you slept? What is the first thing you remember when you woke up? Do you remember anything between sleeping and waking up? Did you dream of anything during the sleep period of your operation? [5].

Our main goal was to compare between groups the remifentanil effect on changes of maternal hemodynamic values during I-D period and on neonatal outcome.

Our second goal was to study the influence of remifentanil on anesthetics consumption

Statistical analyses

The calculation of sample size showed that 15 patients per group would have 90% power with $p < 0.01$ to detect a difference in SAP of 15 mmHg in response to intubation.

Statistical analysis was performed using SSPS statistic package, version 13 (SPSS Inc., Chicago, IL, USA). Normal

Table 1. Parturients' characteristics and surgical details

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p
Age (years) mean ± SD	31.74 ± 4.46	31,22 ± 5.22	30.89 ± 1.04	0.202	0.818
Gestation (weeks) mean ± SD	38.94 ± 0.72	39.04 ± 1.09	39.47 ± 0.9	2.162	0.122
Weight (kg) mean ± SD	77.19 ± 13.27	82.37 ± 9.52	79.26 ± 11.84	2.216	0.918
I-D interval (minutes) mean ± SD	11.22 ± 1.67	10.04 ± 1.81	10.37 ± 1.71	3.639	0.131
U-D interval (seconds) mean ± SD	57.39 ± 18.93	58 ± 14.92	60.42 ± 22.25	0.165	0.848

F – ANOVA; I-D interval induction – delivery interval; U-D interval – uterine incision delivery interval; SD – standard deviation

Table 2. Hemodynamic variables at T0 (basal)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p
SAP mean ± SD	132.42 ± 15.05	132.19 ± 10.53	131.74 ± 10.08	0.018	0.982
DAP mean ±SD	83.90 ± 11.61	83.81 ± 9.78	77.63 ± 12.07	2.246	0.113
MAP mean ± SD	100.48 ± 14.08	100.11 ± 9.28	96.47 ± 11.58	0.818	0.445
HR mean ± SD	101.48 ± 16.01	98.18 ± 13.49	98.84 ± 14.62	0.397	0.674

F – ANOVA; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mmHg); MAP – main arterial pressure (mmHg); HR – heart rate (beats per minute); SD – standard deviation

Table 3. Hemodynamic variables at T1 (after induction)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p	Post hoc
SAP mean ± SD	110.03 ± 14.16	107.14 ± 12.59	116.89 ± 9.93	3.364	0.004	c
DAP mean ± SD	67.93 ± 10.99	71.28 ± 10.51	75.31 ± 14.6	2.313	0.106	
MAP mean ± SD	85.8 ± 13.21	84.22 ± 13.01	91.05 ± 13.17	1.59	0.211	
HR mean ± SD	97.06 ± 9.88	94.7 ± 9.96	103.15 ± 11.64	3.819	0.026	c

F – ANOVA; c – B *vs.* C; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mmHg); MAP – main arterial pressure (mmHg); HR – heart rate (beats per minute); SD – standard deviation

Table 4. Hemodynamic variables at T2 (after intubation)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p	Post hoc
SAP mean ± SD	119.61 ± 13.95	121.89 ± 13.82	149 ± 14.5	29.302	< 0.001	b. c
DAP mean ± SD	75.71 ± 12.93	81.56 ± 10.65	98.21 ± 15.01	18.75	< 0.001	b. c
MAP mean ± SD	91.06 ± 12.6	96.7 ± 12.49	116.68 ± 14.76	23.292	< 0.001	b. c
HR mean ± SD	100.68 ± 8.92	102.41 ± 11.02	109.68 ± 9.61	5.165	0.008	b. c

F – ANOVA; a – A *vs.* B; b – A *vs.* C; c – B *vs.* C; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mmHg); MAP – main arterial pressure (mmHg); HR – heart rate (beats per minute); SD – standard deviation

distribution was evaluated with Kolmogorov–Smirnov test. Analysis of variance (ANOVA) was used for parameters comparison between three groups, with subsequent post hoc analysis. In cases of irregular data distribution, Kruskal–Wallis test was utilized, with subsequent post hoc analysis with Mann–Whitney U–test. The χ^2 test was used to verify the relation between categorical variables. The statistic hypothesis was tested on the significance level for risk of $\alpha = 0.05$; the difference between samples was considered significant if $p < 0.05$.

RESULTS

The patients' characteristics and surgical details are summarized in Table 1; no differences between the groups have been observed.

Tables 2–7 and Figure 1 represent serial hemodynamic values measured at T0 to T5. Baseline (Table 2) and post-induction values (Table 3) did not differ between the groups except for SAP and HR (B *vs.* C), but without clinical significance. After the intubation SAP, DAP, MAP, and HR rose significantly in group C compared to A and B (Table 4).

After skin incision, hemodynamic variables were still significantly higher in group C compared to A, but not compared to B – values in group B began to rise (Table 5).

After peritoneal incision significant difference in SAP, MAP and HR between groups A and C persisted. Significant difference in SAP, DAP and MAP between groups A and B appeared (Table 6).

After delivery, SAP and HR were still significantly higher in group C than in A and SAP and MAP significantly higher in B than in A (Table 7).

BIS values rose significantly after the intubation in all groups compared to pre-intubation values (46–66). In subsequent measurements, BIS values were 58–67 and did not differ between the groups.

Thiopentone dose used for induction in groups A and B was significantly lower than in C (Table 8). Sevoflurane consumption (Table 8) during I-D interval was significantly lower in group A compared to B and C, and lower in B compared to C. After the delivery until the end of the operation sevoflurane, as well as remifentanil consumption was significantly lower in group A compared to B and C (Table 8).

During the operation there were no episodes of hypotension and bradycardia; blood loss and oxytocin consumption where in the average range, with no difference between groups. Maintenance of low remifentanil infusion after the end of surgery allowed smooth emergence from anesthesia without a delay in recovery – patients were extubated within 2–3 minutes after surgery. None of them complained of

Table 5. Hemodynamic variables at T3 (skin incision)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p	Post hoc
SAP mean ± SD	119.06 ± 13.12	124.93 ± 13.09	132.12 ± 8.17	7.948	0.001	b
DAP mean ± SD	75.38 ± 11.74	84.18 ± 10.97	86.84 ± 12.67	6.894	0.002	a. b
MAP mean ± SD	92.83 ± 12.21	99.19 ± 10.81	106.63 ± 10.12	8.951	< 0.001	b
HR mean ± SD	98.81 ± 14.32	102.44 ± 1.89	110.10 ± 11.89	4.520	0.014	b

F – ANOVA; a – A *vs*. B; b – A *vs*. C; c – B *vs*. C; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mmHg); MAP – main arterial pressure (mmHg); HR – heart rate (beat per minute); SD – standard deviation

Table 6. Hemodynamic variables at T4 (peritoneal incision)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p	Post hoc
SAP mean ± SD	118.39 ± 14.28	129.18 ± 15.29	128.94 ± 11.38	5.401	0.006	a. b
DAP mean ± SD	74.65 ± 11.58	84.81 ± 12.56	80.05 ± 13.54	4.855	0.010	a
MAP mean ± SD	91.93 ± 12.84	101.52 ± 14.12	101.05 ± 9.89	5.087	0.009	a. b
HR mean ± SD	96.61 ± 12.76	100.96 ± 12.76	105.89 ± 10.63	3.410	0.038	b

F – ANOVA; a – A *vs*. B; b – A *vs*. C; c – B *vs*. C; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mm Hg); MAP – main arterial pressure (mmHg); HR – heart rate (beats per minute); SD – standard deviation

Table 7. Hemodynamic variables at T5 (delivery)

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	F	p	Post hoc
SAP mean ± SD	116.06 ± 13.93	125.52 ± 9.08	124.31 ± 9.26	5.843	0.004	a. b
DAP mean ± SD	68.55 ± 9.99	76.29 ± 12.82	73.05 ± 16.55	2.663	0.076	
MAP mean ± SD	86.52 ± 11.93	97.37 ± 12.2	94.53 ± 13.53	5.906	0.004	a
HR mean ± SD	91.61 ± 11.59	93.11 ± 11.59	100.95 ± 10.88	3.619	0.032	b

F – ANOVA; a – A *vs*. B; b – A *vs*. C; c – B *vs*. C; SAP – systolic arterial pressure (mmHg); DAP – diastolic arterial pressure (mm Hg); MAP – main arterial pressure (mmHg); HR – heart rate (beats per minute); SD – standard deviation

intraoperative awareness in an interview performed two and again 24 hours after the operation.

Neonatal outcome is presented in Table 9, with no differences between groups in any of estimated variables: 77.4% of neonates in group A, 81.5% in group B and 73.7% in group C started breathing immediately after delivery. The rest of them needed only brief tactile stimulation (12.9%, 7.4%, 15.8%, respectively) or bag mask ventilation (9.7%, 11.1%, 10.5%, respectively) ($\chi_{kw}^2 = 4.365$; p = 0.359%). Umbilical blood gas values were within normal range and did not demonstrate significant differences between groups (Table 10).

DISCUSSION

During the past two decades, numerous authors reported the use of remifentanil during I-D period of caesarean section in order to attenuate maternal stress response to endotracheal intubation and surgical incision. Van de Velde et al. [9], using 0.5 μg/kg remifentanil bolus followed by 0.2 μg/kg/min infusion until delivery, managed to attenuate maternal stress response, but brief respiratory depression was present in half of the newborns. Ngan Kee et al. [7], using 1 μg/kg remifentanil bolus, provided attenuation of maternal BP and HR response, but 10% neonates needed naloxone. Behdad et al. [11] accomplished reduction of SAP and DAP, but not HR, with a remifentanil bolus of 0.5 μg/kg, without neonatal respiratory depression. Draisci et al. [6], using 0.5 μg/kg bolus plus 0.15 μg/kg/min. remifentanil infusion, interrupted at the moment of peritoneal incision, observed partially obtunded neuroendocrine response to surgery, with lower Apgar scores at 1st minute, respiratory depression or required endotracheal intubation in 14% of neonates. It seems that initial 0.5 μg/kg bolus might have been insufficient to accomplish the attenuation of maternal stress response and, on the other hand, that remifentanil infusion, prolonged until peritoneal incision, caused neonatal respiratory depression (the time interval between peritoneal incision and delivery was only 2.8 min.) [6]. Noskova et al. [12], using 1 μg/kg remifentanil, observed higher incidence in lower Apgar scores at 1st minute compared to control, possibly because of short I-D interval (4 min.). Yoo et al. [13] administered 1 μg/kg remifentanil and effectively attenuated hemodynamic response to intubation, but at the expense of maternal hypotension and greater need for neonatal resuscitative measures in the first minutes after delivery. Reduced catecholamine response compared to control was noted at the intubation, but not at delivery, so a single remifentanil dose did not manage to prevent catecholamine rise during the whole period. Hu et al. [10] measured umbilical arterial and venous remifentanil concentration at delivery and proved rapid remifentanil metabolism in fetal circulation, but emphasized that it can be affected by the differences in dosing regimens.

Based on reported data, we created a dosing regimen of 1 μg/kg remifentanil bolus given immediately before the induction, followed by 0.15 μg/kg infusion stopped after skin incision, in attempt to establish both safe and effective regimen that can be used in obstetric clinical practice during I-D period of caesarean section, and compared its maternal and neonatal effects with regimens of sole 1 μg/kg remifentanil bolus and with remifentanil-free control (traditionally performed anesthesia during I-D period). We hypothesized that remifentanil infusion would provide hemodynamic stability during both endotracheal intubation and surgical incision. Earlier infusion interruption than in

Table 8. Consumption of anesthetics

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	χ_{KW}^2/ F*	p	Post hoc
Thiopentone (mg/kg) at induction mean ± SD	4.74 ± 0.64	4.72 ± 0.62	5.63 ± 0.72	13.495*	< 0.001	b. c
Remifentanil consumption: D-end (μg/kg/min) mean ± SD	0.14 ± 0.02	0.17 ± 0.03	0.17 ± 0.05	15.662	< 0.001	a. b
Sevo consumption I-D (vol%)mean ± SD	1.29 ± 0.24	1.5	1.59 ± 0.17	27.890	< 0.001	a. b. c
Sevo consumption D-end (vol%) mean ± SD	0.89 ± 0.1	0.97 ± 0.1	1.01 ± 0.15	11.148	0.004	a. b

F – ANOVA; χ_{KW}^2 – Kruskal–Wallis test; a – A *vs.* B; b – A *vs.* C; c – B *vs.* C; Remifentanil consumption: D-end (μg/kg/min) – remifentanil consumption from the delivery of baby until the end of operation in μg/kg/min; Sevo consumption I-D (vol%) – consumption of sevoflurane during induction delivery period in vol%; Sevo consumption D-end (vol%) – consumption of sevoflurane from the delivery of baby to the end of the operation in vol%; SD – standard deviation

Table 9. Newborns characteristics

Parameter	Group A n = 31	Group B n = 27	Group C n = 19	χ_{KW}^2/F*	p
Ap^1 mean ± SD	8.81 ± 0.55	8.81 ± 0.48	8.63 ± 0.49	2.969	0.227
Ap^5 mean ± SD	9.03 ± 0.31	8.93 ± 0.26	8.89 ± 0.32	2.972	0.226
SpO_2 (%) mean ± SD	95.07 ± 3.37	95.72 ± 2.21	94.61 ± 3.33	3.953	0.307
HR (bpm) mean ± SD	141.48 ± 9.93	138.13 ± 14.35	140.5 ± 12.51	3.423*	0.098

F – ANOVA; χ_{KW}^2 – Kruskal–Wallis test; Ap^1 – Apgar score in 1st minute; Ap^5 – Apgar score in 5th minute; SpO_2 (%) – hemoglobin oxygen saturation (%); HR (bpm) – heart rate (beats per minute); SD – standard deviation

Table 10. Umbilical blood gas values

Parameters	Group A n = 31	Group B n = 27	Group C n = 19	χ_{KW}^2 / F*	p
venous pH mean ± SD	7.3 ± 0.02	7.32 ± 0.03	7.32 ± 0.03	3.879	0.144
venous BD mmol/l mean ± SD	5.06 ± 2	4.07 ± 1.61	5.08 ± 1.38	2.757*	0.070
venous lactate (mmol/l) mean ± SD	1.28 ± 0.24	1.31 ± 0.25	1.21 ± 0.21	0.836*	0.438
arterial pH mean ± SD	7.27 ± 0.02	7.28 ± 0.02	7.28 ± 0.08	2.162	0.339
arterial BD (mmol/l) mean ± SD	4.19 ± 2.2	4.27 ± 1.66	4.41 ± 1.14	0.082*	0.921
arterial lactate (mmol/l) mean ± SD	1.3 ± 0.33	1.3 ± 0.36	1.39 ± 0.27	0.836*	0.438

F – ANOVA; χ_{KW}^2 – Kruskal–Wallis test; SD – standard deviation

previous studies (after skin incision instead of at peritoneal incision or even at delivery) should leave enough time for remifentanil redistribution and metabolism in fetal circulation, thus diminishing the probability of neonatal respiratory depression.

Hemodynamic variables measured after the intubation in groups A and B were significantly lower than in group C. Therefore, both regimens attenuated cardiovascular response to endotracheal intubation, which is in accordance with previous reports [4, 9, 12–16]. The next measurement, performed after skin incision, already showed the difference: the significant difference in SAP, DAP, MAP, and HR between groups B and C disappeared, but persisted in A compared to C. At the time of peritoneal incision and at the delivery measured hemodynamic variables were significantly lower in group A compared to both C and B group. It appears that remifentanil bolus plus infusion regimen (group A) effectively blunted cardiovascular response during entire I-D period whereas sole remifentanil bolus (group B), was not effective enough to provide hemodynamic stability in a period following intubation.

Synergism between remifentanil and anesthetics has been described in numerous studies [17, 18, 19]. Our results are in agreement with those data. Thiopentone dose was significantly lower in remifentanil groups than in control. Prolonged remifentanil infusion in group A provided significantly diminished sevoflurane requirements during I-D period, and also during the rest of operation. We believe that adequate analgesia, achieved in group A before the start of noxious stimulation and kept during surgical incision (preemptive approach), caused lower remifentanil consumption in a period from delivery until the end of the operation.

In our research, remifentanil administration did not affect BIS values, which is in agreement with other reports [5, 6, 13]. BIS values are the reflection of hypnotic drugs action on cerebral cortex, whereas opioids act primarily on subcortical level, and their sedative effects cannot be detected by BIS monitoring. When appropriate BIS level during remifentanil/sevoflurane based anesthesia is considered, it is emphasized that attempts to maintain the target BIS of 40–60 would lead to an excessively deep level of anesthesia and 50–150% higher end-tidal sevoflurane concentration than actually needed [20]. BIS values in our research remained 58–68 throughout the whole operation. Nevertheless, even with reduced anesthetic consumption in remifentanil groups (especially in group A), the achieved hypnotic state was adequate, estimated by the absence of somatic, autonomic and hemodynamic responses to noxious stimuli, but also by the absence of explicit memory of operation period.

Our results did not demonstrate negative remifentanil effects on neonatal outcome. Opposite to the results from mentioned studies all neonatal Apgar scores at 1st minute were ≥ 8; oxygen saturation and HR were within normal range and without differences between groups [6, 7, 9, 12, 13]. Majority of neonates started breathing within a few seconds after delivery; the rest of them needed only brief (up to one minute) tactile stimulation or bag mask ventilation. Similarly to other studies, we did not find differences

in umbilical blood gas analysis, and all values were within normal range [6, 7, 12, 13, 21, 22].

CONCLUSION

Our dosing regimen of remifentanil bolus given at the induction, followed by infusion interrupted after skin incision, effectively prevented significant rise in BP and HR during entire I-D period without compromising neonatal wellbeing and significantly diminished anesthetics consumption, so it can be considered effective as well as safe to use during I-D period of caesarean section.

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Ефекат примене ремифентанила током царског реза на хемодинамику породиље и неонатални исход – поређење два режима дозирања

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САЖЕТАК

Увод/Циљ Циљ рада је приказати ефекте два режима дозирања ремифентанила на породиљу и неонатус примењеног током царског реза у периоду од увода у анестезију до порођаја, у циљу супримирања матерналног кардиоваскуларног одговора на хируршки стрес.

Методе Седамдесет седам породиља *ASA* I-II статуса је методом случајног избора подељено на три групе: *A* – 31 породиља која је непосредно пре увода у анестезију примила 1 *μg/kg* болус ремифентанила, који је настављен инфузијом од 0,15 *μg/kg/min.* прекинутом по начињеном резу коже; *B* – 27 породиља које су непосредно пре увода у анестезију примиле болус ремифентанила од 1 *μg/kg*; *C* – 19 породиља које нису примиле ремифентанил пре рађања неонатуса. Упоређиване су промене хемодинамике и биспектралног индекса у периоду од увода до екстракције, интраоперативна потрошња анестетика и ремифентанила и неонатални исход.

Резултати Хемодинамски одговор на интубацију је супримиран ($p < 0,001$) у групама *A* и *B* у односу на *C*. Хемодинамски одговор на инцизију коже, инцизију перитонеума и на екстракцију је значајно супримиран у групи *A* у поређењу са групама *B* и *C*. Потрошња тиопентона је смањена ($p < 0,001$) у групама *A* и *B* у поређењу са групом *C*. Потрошња севофлурана и ремифентанила је била мања у групи *A* у поређењу са групама *B* и *C* ($p < 0,001$). Апгар скорови у првом минуту су код свих неонатуса били ≥ 8; није било разлика у фреквенци рада срца, сатурацији хемоглобина кисеоником и вредностима гасних анализа умбиликалне крви (све у референтним границама).

Закључак Болус ремифентанила 1 *μg/kg* апликован на уводу у анестезију и настављен инфузијом од 0,15 *μg/kg/min.* до инцизије коже успешно је супримирао матернални хемодинамски стресни одговор на хируршки стрес током целог периода од увода до екстракције, смањио потрошњу анестетика и аналгетика, при томе без штетних ефеката по неонатусе, па се може сматрати и ефикасним и сигурним режимом за коришћење од увода у анестезију до рађања неонатуса.

Кључне речи: анестезија; акушерска; ремифентанил

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association of advanced oxidation protein product, thiobarbituric acid reactive substances and total sulfhydryl groups with retinal blood vessels' caliber

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SUMMARY

Introduction/Objective Intensive oxidative stress is proven in patients with diabetes mellitus and important in the development of a microvascular complication of type 2 diabetes mellitus.
The aim of the study was to investigate the relationship between morphometric parameters of retinal blood vessels in patients with diabetic retinopathy (DR) and the levels of parameters of oxidative stress: advanced oxidation protein product (AOPP), thiobarbituric acid reactive substances (TBARS), and total sulfhydryl (SH) groups in blood samples.
Methods The patients (the group with DR and controls) were sex- and age-matched. Glycaemia, hemoglobin A1C HbA1C, total cholesterol and its fractions, and triglycerides were measured in blood samples. AOPP and total SH groups were determined in the plasma by specific methods. Modification of the thiobarbituric acid method was used for the determination of TBARS.
The number and diameter of retinal blood vessels, as morphometric parameters on digital retinal photography, was determined by using the ImageJ software. Student's t-test was used as the statistical method for the evaluation of differences between the morphometric and blood test parameters. The significance of differences in morphometric parameters of retinal blood was establish by one-way ANOVA.
Results Significantly higher levels of parameters of oxidative stress (AOPP and TBARS) were in the group of patients with DR than in the controls. This difference was also present among the patients with mild and severe forms of DR (AOPP F 77.03, $p < 0.001$) (TBARS F 63.28, $p < 0.001$).
The diameter of retinal blood vessels correlated with levels of AOPP, but only in patients with mild DR.
Conclusion Parameters of oxidative stress, AOPP and TBARS, may be important for the follow-up of DR. In early stages in diabetic retinopathy, AOPP can be a valuable biomarker.
Keywords: diabetic retinopathy; oxidative stress; retinal vessels

INTRODUCTION

A vision-threatening microvascular complication of diabetes reported in about one-third of patients is diabetic retinopathy (DR) [1].

According to American Diabetes Association and Diabetic Retinopathy Guidelines, DR can be categorized as early non-proliferative diabetic retinopathy (mild NPDR), moderated and severe, or pre-proliferative diabetic retinopathy (PPDR), and proliferative diabetic retinopathy (PDR) [1]. Microaneurysms and blot hemorrhages are clinical sings of mild non-proliferative DR. In the middle stages, NPDR hard exudates, maculopathy, venous changes, retinal capillary loss and ischemia, cotton wool or soft exudates, dot, blot spots, and extensive intraretinal hemorrhages are present [2]. Neovascularization, preretinal and vitreous hemorrhage, fibrovascular proliferation, and retinal detachments are present in patients with PDR [2].

Retinal tissue is rich in polyunsaturated fatty acids, is directly exposed to UV radiation and has high demand for energy, which makes it prone to oxidative stress. Oxidative stress is proven in patients with DM and is also important in the development of a microvascular complication of DMT2 [3–6].

The objective of this paper was to investigate the correlation of the number and the diameter of retinal blood vessels as morphometric parameters and oxidative stress parameters – oxidation protein product (AOPP), thiobarbituric acid reactive substances (TBARS) and total sulfhydryl (SH) groups, as the parameter of antioxidative defense in patients with diabetic retinopathy.

METHODS

Subjects

The study included 51 Caucasian patients. Seventeen patients (nine males and eight females) were with mild NPDR and nine patients (four

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females and five males) had PPDR. The control group included 25 healthy individuals (14 males and 11 females). Family history of diabetes was negative. The excluding criteria were intraocular inflammatory diseases (scleritis, uveitis), glaucoma and age-related macular degeneration, smoking, use of angiotensin receptor blockers, antioxidants or mineral supplements, any previous ophthalmic surgical or laser interventions. The study was performed at the Clinic for Eye Diseases, at the Center for Biochemical Research of the Clinical Centre Niš, and the Department of Biochemistry, Faculty of Medicine, University of Niš, Serbia. All the patients were informed about the methods and the aim of the study, and their written informed consent to participate was obtained. The study was performed in agreement with the rules and was approved by the Internal Ethic Committee of the Faculty of Medicine in Niš.

In all subjects, the ophthalmic examination attains the following: best corrected visual acuity, tonometry, anterior segment and posterior segment examination by indirect ophthalmoscopy, fundus photography, and fluorescein angiography. Fundus photography and fluorescein angiography were done in all the patients with DR, under the same conditions, using the same digital fundus camera and by the same ophthalmologist. ETDRS classification was used for the staging of the DR [2].

Blood chemistry analysis

Glycaemia, HbA1C, total cholesterol and its fractions (LDL-C and HDL-C), and triglycerides were measured in blood samples with AU680 clinical chemistry analyzer (Olympus Corporation, Tokyo, Japan). The samples were collected in early morning on an empty stomach.

AOPP was determined in the plasma using the method of Witko-Sarsat et al. [7]. The concentration of AOPP groups was expressed in μmol/L.

Total SH concentration was determined by using 5-5'-dithiobis-(2-nitrobenzoic acid) [8]. Absorbance was measured at 412 nm against blank samples and expressed as mmol/L. Concentrations of SH groups were expressed in μmol/L.

TBARS were determined by the modification of the TBA method [9]. The concentration of TBARS was expressed in μmol/L.

Morphometric analysis

Morphometric analysis of the digital fundus photography was preformed using the ImageJ software in all examined participants. Both eyes in each patient were analyzed (Figures 1 and 2). According to the manufacturer instructions, spatial calibration for the magnification of retinal digital camera (1 pixel = 17.7 μm) was used. In the first phase of the morphometric analysis, the optic disc Ferret's diameter (D_F), circularity, and centroid were measured. Subsequently, in the second phase we applied the "concentric circles" plugin in order to divide retinal images into five concentric zones whose center was the centroid of the optic disc (Figure 1). The first concentric area was

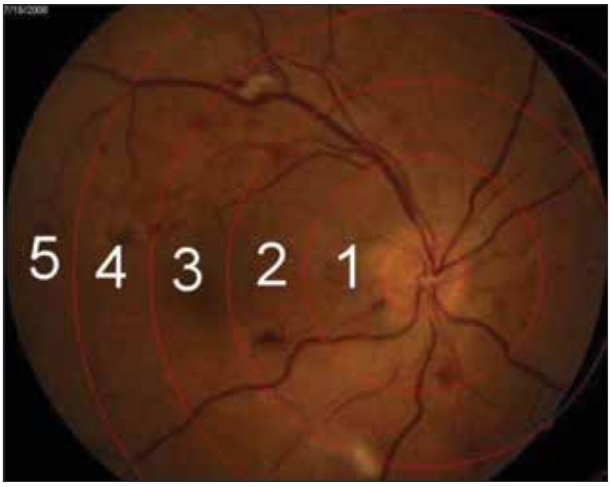

Figure 1. Digital fundus photography with concentric zones – the right eye

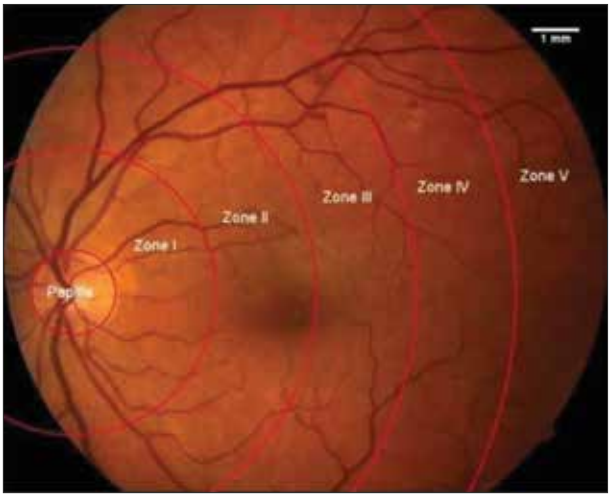

Figure 2. Digital fundus photography with concentric zones – the left eye

the optic papilla and the area next to it was marked as the first zone (zone I). Other zones (zones II–V) were marked according to the gradual increase of their distance from the optic disc. The zones were constructed as equal and their size in different patients depended on the optic disc location in the retinal images. Macular region was located in zones II and III. The number of retinal blood vessels in each retinal zone, including the optic disc was established with the "cell counter" plugin. In the case of blood vessel bifurcations, two newly formed blood vessels were counted as separate vessels. The outer diameter of all counted blood vessels (D_{BW}) in one zone was measured at three different localizations in each of them, and then the mean value was calculated. The same method was used in the study by Cekić et al. [10].

Statistical method

Statistical package NCSS PASS 2007 (National Council for the Social Studies, USA) was used for the statistical analysis. Kruskal–Wallis one-way ANOVA test and Dunn's post-hoc test were used to compare median values between

Table 1. Mean values of measured parameters in blood of evaluated groups

Parameter	Group	n	Mean	SD	F	p
Age	Control	25	52.12	6.19	2.65	n.s.
	Mild NPDR	17	57.71	7.90		
	PPDR	9	55.78	11.61		
HbA1c (%)	Control	25	5.08	0.52	39.52	< 0.0001
	Mild NPDR	17	7.99	1.52		
	PPDR	9	8.64	2.07		
SH	Control	25	300.96	63.52	24.08	< 0.0001
	Mild NPDR	17	401.83	50.18		
	PPDR	9	267.89	27.04		
AOPP	Control	25	31.11	4.06	77.03	< 0.0001
	Mild NPDR	17	47.51	10.82		
	PPDR	9	87.09	22.92		
TBARS	Control	25	12.08	1.77	63.28	< 0.0001
	Mild NPDR	17	16.15	1.03		
	PPDR	9	20.71	3.68		

HbA1C – hemoglobin A1C; SH – total sulfhydryl groups; AOPP – advanced oxidation protein product; TBARS – thiobarbituric acid reactive substances; NPDR – non-proliferative diabetic retinopathy; PPDR – pre-proliferative diabetic retinopathy;

Control *vs*. I, $p < 0.0001$; mild NPDR *vs*. PPDR, $p < 0.0001$; control *vs*. II, $p < 0.000$

the groups, while Mann–Whitney U-test was used in case of two groups.

Correlations between parameters were established by Spearman's rho (ρ).

RESULTS

The patients were classified into two groups according to changes detected by indirect ophthalmoscopy, fundus photography, and fluorescein angiography. ETDRS classification was used. In patients with mild DR, a small number of microaneurysms was detected. Microaneurysms, different forms of hemorrhage (dot, blot spots, and intraretinal hemorrhages) and cotton walls, venous bleeding and intraretinal vascular abnormalities (IRMAs) in two or more quadrants were detected in the group of patients with PPDR.

The mean age of the examined group of patients and results of the median values of evaluated parameters of blood are presented in Table 1. Median duration of DMT2 in the two groups of patients was not statistically different ($Z = 1.89$, $p = 0.06$) (Table 1). The levels of hemoglobin A1C (HbA1C) were higher in the group with very severe form and were significantly higher than those in the group with mild NPDR ($Z = 2.26$, $p < 0.001$).

The values for AOPP and TBARS as biomarkers of oxidative stress, and total SH groups as parameter of antioxidative defense are presented in Table 1. The values for SH group were higher in group with mild NPDR than in controls and group of patients with PPDR (F 24.08, $p < 0.001$).

Levels of AOPP were significantly higher in group of patients with DR than in controls, as well as among the two different groups with different form of DR (F 77.03, $p < 0.001$) as well as levels of TBARS (F 63.28, $p < 0.001$).

The results of the morphometric analysis were used for cluster analysis (k-means method) and mean values are given in Table 2. These tables also present the results of the Student's t-test.

The values of the average number of observed blood vessels increased from the optic disc towards zone III and then decreased gradually towards zone V. The average number of blood vessels per zone showed a similar trend on the left side in the group of patients with mild NPDR. In the group with PPDR in zones I, II, and III there was a significantly higher average number of blood vessels than in the optic disc and zones IV and V.

The average blood vessel outer diameter decreased from the optic disc towards zone V, and this decrease was significant on the right side in mild NPDR and in PPDR.

The outer diameter of the blood vessels in zones III, IV, and V was significantly ($p < 0.05$) lower than in the optic disc. This parameter showed a similar trend in mild NPDR and PPDR. On both eyes and in both groups of examined patients (NPDR and PPDR), the outer diameter of blood vessels decreased from the optic disc towards zone V.

Finally, correlation analysis revealed that the outer diameter positively correlated with the levels of AOPP (Table 3). This correlation was present on the optic disc and in zones I–III and only for patients with the early or mild form of NPDR. The levels of SH groups also had similar correlation with morphological parameters of blood vessels but not in all the zones and only in the group of patients with PPDR (Table 4). This correlation was not present for levels of TBARS (Tables 3 and 4).

DISCUSSION

Oxidative stress is proven in patients with DM and in pathogenesis of the microvascular complication [11, 12]. The objective of the present study is to investigate the correlation between the levels AOPP and TBARS with the severity of the disease and morphometric parameters of retinal blood vessels.

in plasma of examined patients with DR, significantly higher levels of AOPP and TBARS were present, which correlated positively with the progression of DR (Table 1). Correlation analysis revealed that AOPP and the diameter of retinal blood vessels correlated positively in patients with mild, early stage of DR (Table 3). According to this result, AOPP maybe a biomarker of early changes in DR.

In diabetes, the formation of AOPP is induced by intensified glycoxidation processes, oxidant–antioxidant imbalance, and coexisting inflammation. The role of AOPP in pathogenesis of DR could be explained by its structural and biological similarity with advanced glycation product (AGE) [13]. Also, it is proposed that AOPP expresses proinflammatory activities [13, 14].

AOPP accumulation contributes to DR thought direct tissue damage effects, as well as thought the activation of specific AGE receptors (RAGE) [13, 14, 15]. RAGE activation induces permeability of microvascular endothelial cells and the production of reactive oxygen species

Table 2. Number and diameter of retinal blood vessels on the right and the left eye in the examined zones

Zone	Parameter	Group	n	Right eye	p	Left eye	p
				$\bar{x} \pm SD$		$\bar{x} \pm SD$	
Optic disc	Number	Control	25	18.34 ± 3.33		18.44 ± 3.15	
		Mild NPDR	17	17.77 ± 3.77[a]	0.05	16.33 ± 3.66[a]	0.05
		PPDR	9	14.01 ± 3.49[a,b]	0.05	14.56 ± 3.18[a,b]	0.05
	DBV (μm)	Control	25	74.89 ± 10.35		74.33 ± 8.04	
		Mild NPDR	17	83.66 ± 5.66[a]	0.05	85.33 ± 9.17[a]	0.05
		PPDR	9	94.77 ± 12.28[a,b]	0.05	93.05 ± 14.17[a,b]	0.05
Zone I	Number	Control	25	29.87 ± 5.610		29.989 ± 4.982	
		Mild NPDR	17	27.95 ± 5.111[a]	0.05	30.000 ± 6.922[a]	0.05
		PPDR	9	24.11 ± 4.106[a,b]	0.05	26.333 ± 4.472[a,b]	0.05
	DBV (μm)	Control	25	74.287 ± 8.317		76.579 ± 7.563	
		Mild NPDR	17	79.043 ± 6.660[a]	0.05	79.455 ± 8.551[a]	0.05
		PPDR	9	93.300 ± 10.643[a,b]	0.05	91.888 ± 12.211[a,b]	0.05
Zone II	Number	Control	25	43.760 ± 8.828		40.67 ± 8.079	
		Mild NPDR	17	39.294 ± 2.289[a]	0.05	38.29 ± 8.308[a]	0.05
		PPDR	9	28.667 ± 7.382[a,b]	0.05	30.00 ± 7.104[a,b]	0.05
	DBV (μm)	Control	25	64.194 ± 8.052		64.312 ± 7.166	
		Mild NPDR	17	68.537 ± 7.768[a]	0.05	66.964 ± 8.262[a]	0.05
		PPDR	9	83.959 ± 11.610[a,b]	0.05	85.284 ± 14.971[a,b]	0.05
Zone III	Number	Control	25	44.42 ± 7.070		42.97 ± 10.725	
		Mild NPDR	17	41.65 ± 11.096[a]	0.05	39.75 ± 9.333	NS
		PPDR	9	26.01 ± 11.342[a,b]	0.05	25.11 ± 9.033[a,b]	0.05
	DBV (μm)	Control	25	59.389 ± 8.591		60.269 ± 10.111	
		Mild NPDR	17	62.994 ± 8.321[a]	0.05	59.733 ± 7.566	NS
		PPDR	9	77.766 ± 10.042[a,b]	0.05	76.198 ± 13.220[a,b]	0.05
Zone IV	Number	Control	25	36.54 ± 7.832		31.32 ± 7.966	
		Mild NPDR	17	35.86 ± 8.377[a]	0.05	34.17 ± 10.979[a]	0.05
		PPDR	9	23.45 ± 11.22[a,b]	0.05	19.00 ± 7.882[a,b]	0.05
	DBV (μm)	Control	25	55.60 ± 6.683		57.676 ± 11.333	
		Mild NPDR	17	56.376 ± 4.518[a]	0.05	57.767 ± 10.152[a]	0.05
		PPDR	9	75.043 ± 8.860[a,b]	0.05	71.884 ± 14.044[a,b]	0.05
Zone V	Number	Control	25	21.40 ± 5.991		16.76 ± 6.023	
		Mild NPDR	17	21.55 ± 5.666	NS	15.47 ± 6.135[a]	0.05
		PPDR	9	13.33 ± 4.242[a,b]	0.05	10.78 ± 4.764[a,b]	0.05
	DBV (μm)	Control	25	56.110 ± 6.406		61.805 ± 15.052	
		Mild NPDR	17	56.555 ± 6.731	NS	59.487 ± 10.324[a]	0.05
		PPDR	9	75.012 ± 11.334[a,b]	0.05	74.795 ± 17.345[a,b]	0.05

DBV – blood vessel diameter; NPDR – non-proliferative diabetic retinopathy; PPDR – pre-proliferative diabetic retinopathy;

[a]$p < 0.05$ *vs*. controls;
[b]$p < 0.05$ *vs*. mild NPDR

Table 3. Correlation between the number and the diameter of retinal blood vessels and AOPP, SH groups and TBARS in mild NPDR patients

		Right eye													
Parameter		RPC	RPD_F	RPN_{BV}	RPD_{BV}	$RZ1N_{BV}$	$RZ1D_{BV}$	$RZ2N_{BV}$	$RZ2D_{BV}$	$RZ3N_{BV}$	$RZ3D_{BV}$	$RZ4N_{BV}$	$RZ4D_{BV}$	$RZ5N_{BV}$	$RZ5D_{BV}$
SH	R	0.143	0.252	-0.513	0.074	-0.08	0.27	-0.256	-0.285	-0.035	-0.257	0.116	-0.162	0.216	0.022
	p	0.585	0.33	0.035	0.777	0.76	0.294	0.321	0.268	0.893	0.32	0.658	0.535	0.404	0.933
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17
AOPP	R	-0.509	0.287	-0.135	-0.492	-0.198	-0.493	0.016	-0.181	0.475	-0.151	0.153	-0.372	0.072	-0.084
	p	0.037	0.265	0.605	0.045	0.445	0.045	0.953	0.047	0.054	0.043	0.557	0.142	0.784	0.749
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17
TBARS	R	-0.342	0.37	-0.193	-0.231	0.374	0.032	0.135	-0.104	0.222	0.058	-0.302	0.128	-0.258	0.104
	p	0.178	0.144	0.459	0.373	0.139	0.904	0.607	0.692	0.391	0.826	0.238	0.625	0.317	0.69
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Left eye															
Parameter		LPC	LPD_F	LPN_{BV}	LPD_{BV}	$LZ1N_{BV}$	$LZ1D_{BV}$	$LZ2N_{BV}$	$LZ2D_{BV}$	$LZ3N_{BV}$	$LZ3D_{BV}$	$LZ4N_{BV}$	$LZ4D_{BV}$	$LZ5D_B$	$LZ5N_{BV}$
SH	R	0.086	0.009	-0.241	0.108	-0.166	0.238	-0.351	0.369	-0.361	0.412	-0.374	0.507	-0.237	0.153
	p	0.743	0.972	0.352	0.681	0.525	0.358	0.168	0.145	0.155	0.1	0.139	0.038	0.359	0.556
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17
AOPP	R	-0.565	0.512	-0.286	-0.044	-0.362	0.402	-0.34	0.333	-0.06	-0.285	0.103	-0.007	-0.067	0.143
	p	0.018	0.036	0.266	0.05	0.153	0.01	0.182	0.052	0.82	0.267	0.693	0.979	0.798	0.585
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17
TBARS	R	-0.548	0.409	-0.141	0.093	0.028	0.052	-0.44	0.053	-0.368	-0.206	-0.327	0.131	-0.255	0.309
	p	0.023	0.103	0.59	0.723	0.914	0.844	0.077	0.839	0.146	0.428	0.2	0.617	0.324	0.228
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17

SH – total sulfhydryl groups; AOPP – advanced oxidation protein product; TBARS – thiobarbituric acid reactive substances; NPDR – non-proliferative diabetic retinopathy; PPDR – pre-proliferative diabetic retinopathy; PC – papillar circularity; PD_F – papillar diameter; PN_{BV} – papillar number of blood vessels; PD_{BV} – diameter of blood vessels on papilla / optic disc; L – left eye; R – right eye; Z – zone; N_{BV} – number of blood vessels; D_{BV} – diameter of blood vessels

Table 4. Correlation between the number and the diameter of retinal blood vessels eye and AOPP, SH groups, and TBARS in PPDR patients

Right eye															
Parameter		RPC	RPD_F	RPN_{BV}	RPD_{BV}	$RZ1N_{BV}$	$RZ1D_{BV}$	$RZ2N_{BV}$	$RZ2D_{BV}$	$RZ3N_{BV}$	$RZ3D_{BV}$	$RZ4N_{BV}$	$RZ4D_{BV}$	$RZ5N_{BV}$	$RZ5D_{BV}$
SH	R	0.654	0.093	0.186	-0.008	0.457	0.111	0.281	0.378	0.18	0.268	0.396	-0.327	-0.131	0.587
	p	0.056	0.812	0.631	0.984	0.216	0.777	0.464	0.316	0.644	0.485	0.292	0.391	0.737	0.097
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
AOPP	R	0.032	0.151	0.332	-0.095	-0.436	0.441	-0.585	0.319	-0.496	-0.089	-0.531	0.482	-0.326	0.231
	p	0.935	0.697	0.383	0.807	0.241	0.234	0.098	0.402	0.174	0.82	0.141	0.189	0.392	0.55
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
TBARS	R	-0.068	0.151	0.367	-0.32	0	0.059	-0.335	0.281	-0.458	0.385	-0.309	0.362	-0.41	0.284
	p	0.861	0.699	0.331	0.401	1	0.879	0.379	0.464	0.215	0.307	0.418	0.339	0.273	0.459
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Left eye															
Parameter		LPC	LPD_F	LPN_{BV}	LPD_{BV}	$LZ1N_{BV}$	$LZ1D_{BV}$	$LZ2N_{BV}$	$LZ2D_{BV}$	$LZ3N_{BV}$	$LZ3D_{BV}$	$LZ4N_{BV}$	$LZ4D_{BV}$	$LZ55N_{BV}$	$RZ5D_{BV}$
SH	R	0.36	-0.083	0.687	-0.63	0.657	-0.632	0.395	-0.764	0.67	-0.82	0.45	-0.69	-0.024	-0.296
	p	0.342	0.832	0.041	0.069	0.055	0.068	0.293	0.017	0.048	0.007	0.225	0.04	0.951	0.439
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
AOPP	R	0.281	-0.307	0.001	-0.011	-0.392	-0.036	-0.215	0.184	-0.389	0.388	-0.27	0.007	-0.001	-0.184
	p	0.464	0.422	0.997	0.977	0.297	0.926	0.578	0.635	0.301	0.302	0.483	0.987	0.999	0.636
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
TBARS	R	-0.232	0.241	0.224	-0.234	-0.3	-0.325	-0.291	-0.039	-0.263	-0.112	0.079	-0.208	-0.232	-0.409
	p	0.548	0.533	0.563	0.544	0.433	0.393	0.448	0.921	0.495	0.774	0.839	0.591	0.548	0.275
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9

SH – total sulfhydryl groups; AOPP – advanced oxidation protein product; TBARS – thiobarbituric acid reactive substances; NPDR – non-proliferative diabetic retinopathy; PPDR – pre-proliferative diabetic retinopathy; PC – papillar circularity; PD_F – papillar diameter; PN_{BV} – papillar number of blood vessels; PD_{BV} – diameter of blood vessels on papilla / optic disc; L – left eye; R – right eye; Z – zone; N_{BV} – number of blood vessels; D_{BV} – diameter of blood vessels

(ROS). Endothelial damage due to accumulation of AGE, activation of PKC, increased expression of vascular endothelial growth factor and intercellular adhesion molecule (ICAM-1), and increases in ROS lead to the expression of endothelial nitrite oxide synthetases. RAGE activation subsequently evokes fibrogenic reaction [11]. Thickening of the basement membrane coupled with its increased permeability, loss of pericytes leading to diminished vessel wall tone, and development of protruding microaneurysms, as well as proliferation of mesangial cells and consequent obstruction and obliteration of capillaries are results of all of these processes.

The results of morphological changes in our examined patients have shown the outer diameter of blood vessels decreased significantly with the progression of DR (Table 2). The same results are presented in a study by Cekić et al. [10]. The remodulation and regression of vascular net in DR has been in focus of many different studies [12, 13]. Formation of peroxynitrite due to a reaction between ROS and nitric oxide further causes endothelial dysfunction. Increased apoptosis of retinal capillary cells is a result of the damage of the mitochondrial lipid membrane by ROS. Increased nitrate stress in retinal vascular cells, via the activation of nuclear transcriptional factor, NF-kB by AGE, leads to apoptosis of retinal pericytes [12, 16]. Our results have shown that the levels AOPP correlate with the severity of DR.

the levels of TBARS are elevated in both groups of patients with DR and correlate with the severity of disease (Table 1). However, the levels of this parameter of oxidative stress did not show a correlation with retinal blood vessels in our study. Similar results are presented in the study

conducted by Ruia et al. [17]. TBARS serve as potential biomarkers for DR.

The antioxidant status of a diabetic patient has an important role in producing oxidative stress and the development of vascular complications in patients with DM. The reports of antioxidants and antioxidant enzymes in DR patients are contradictory [4, 5, 6, 18]. The total thiol levels as a marker of antioxidant status in diabetics has shown to be significantly decreased in patients with DR. In our study, the levels of total SH group in serum were higher in NPDR, and significantly lower in PPDR. An inverse correlation between the level of HbA1C and total SH groups in patients with a moderated form of DR indicate a reduction in antioxidant status in poorly controlled patients. Sharma et al. [19] have demonstrated that decreased GSH levels in patients with PDR are associated with *in vivo* structural changes of the retina. These results correlate with our own, but the precise mechanisms are still unclear. Therefore, the levels of total SH groups could be predictive for the development of DR and its progression.

CONCLUSION

These findings suggest that AOPP and TBARS can be used as a biomarker for DR and its progression. The levels of AOPP correlate with the diameter of retinal blood vessels in the early stage of DR – hence, AOPP may be a parameter of the early stage of DR.

Limitations of this study that should be noted are the following: only Caucasian patients were included, the influence of local and ocular factors on retinal blood vessel caliber could not be avoided. More precise medical imaging and correlation with the studied parameter are needed.

Conflict of interest: None declared.

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Утицај продуката узнапредовале оксидације, супстанци реактивних са тиобарбитурном киселином и укупних сулфхидрилних група на дијаметар крвних судова ретине

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САЖЕТАК

Увод/Циљ Интензивни оксидативни стрес утврђен је код болесника са дијабетесом мелитусом и важан је код развоја микроваскуларних компликација дијабетеса мелитуса типа 2.

Циљ нашег рада био је утврђивање везе између нивоа параметара оксидативног стреса, продуката убрзане оксидације протеина (*AOPP*) и тиобарбитурно реактивних супстанци (*TBARS*) и параметра антиоксидативне заштите укупне сулфхидрилне групе у узорцима крви са морфометријским параметрима код испитаника са дијабетичном ретинопатијом (ДР).

Метод Испитаници подељени на групу болесника са ДР и контролну групу били су усклађени по полу и узрасту. Лабораторијске анализе крви обухватале су одређивање гликемије наште, *HbA1C*, укупног холестерола, фракција *LDL*, *HDL*, триглицерида. *AOPP* и сулфхидрилне групе одређивани су у плазми испитаника. *TBARS* одређиван је модификованом методом тиобарбитурне киселине.

За морфометријску анализу крвних судова ретине, број и дијаметар, коришћен је софтвер *ImageJ* за анализу дигиталне фотографије очног дна. За статистичку анализу биохемијских и морфометријских параметара коришћен је Студентов *t*-test, а једнофакторска анализа варијансе (*one-way ANOVA*) за утврђивање статистички значајне разлике.

Резултати Вредности *AOPP* и *TBARS* биле су статистички значајно више у групи испитаника са узнапредовалом ДР (*AOPP* F 77,03, $p < 0{,}001$) (*TBARS* F 63,28, $p < 0{,}001$). Вредности *AOPP* корелирале су са вредностима дијаметра крвних судова.

Закључак Вредности *AOPP* и *TBARS* могу бити параметри праћења развоја ДР, а вредности *AOPP* могу бити биомаркер раног стадијума ДР.

Кључне речи: дијабетична ретинопатија; оксидативни стрес; крвни судови ретине

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Quality of life in patients with laryngeal cancer before and after surgery

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SUMMARY

Introduction/Objective Assessment of the Quality-of-life questionnaires was filled out before and after surgery by patients with laryngeal cancer hospitalized in the Otolaryngology, Head and Neck Surgery Department, qualified for surgical treatment.

Methods Fifty-four patients with laryngeal cancer in T3 and T4 stages who were qualified for total laryngectomy were asked to fill out the EORTC QLQ-30 and H&N30 modules before and a few years after surgical treatment.

Results The quality of life of the hospitalized patients increased after surgery. The level of pain after surgery decreased and was statistically significant ($p = 0.025$). In the study group, 90.6% of patients survived five years after surgery.

Conclusion The quality of life in patients with laryngeal cancer improved in the domain of pain. Further research should be conducted on a larger group of patients. Future results could provide useful material for analysis regarding the benefits for the patient that may be relevant to a decision to consent to the proposed treatment and the choice of its type.

Keywords: quality of life; laryngeal cancer; follow-up; laryngectomy

INTRODUCTION

According to the World Cancer Research Fund International, 1.1% of all cancers is laryngeal cancer, the most common cancer of the head and neck neoplasms [1]. Males are affected more often than females. Primary risk factors are tobacco smoking, alcohol consumption, and human papilloma virus infection.

Laryngeal cancer is generally squamous cell carcinoma. Symptoms mostly begin with hoarseness in the voice but also may include a lump, sore throat, and swallowing difficulties. Treatment methods include surgery, radiotherapy, and chemotherapy. Infiltration of the laryngeal cartilages is an indication for a procedure called laryngectomy, consisting of total excision of the larynx [2]. Larynx plays the critical role in physiologic functions such as voice production, respiration, airway protection and swallowing – this is why total laryngectomy may significantly affect the patients' quality of life (QoL). Lately, the medical community puts great emphasis on the quality of life, which is why more and more studies on the QoL are conducted.

The World Health Organization defines the QoL as the person's perception of his or her individual daily life and position. It takes into consideration the context of culture, the personal relation to the goals, the situation in which the person lives, expectations, and concerns. In healthcare, the QoL is an assessment of how aspects of an individual's life can be affected by disease or disability [3]. Measuring the QoL gives an enormous amount of information that should be considered in the selection of a treatment method. For example, organ preservation is not necessarily needed to have better QoL. Measuring the QoL also determines how important survival is after treatment [4].

Two types of QoL assessment tools exist – general and specific. QoL recorded with the impact of disease in particular is called general. Specific scales assess the QoL by taking into account a specific group of diseases, a single disease, or a single symptom [5].

In our earlier studies, all PUBMED articles about the QoL in patients with laryngeal cancer were reviewed, and different measuring tools were identified. The European Organization for Research and Treatment of Cancer (EORTC) questionnaire general module (QLQ C-30) and head and neck module (QLQ-H&N35) turned out to be the most commonly used tools to assess the QoL in patients with different stages of laryngeal cancer or to compare treatment methods. Therefore, we decided to use this questionnaire in the current study.

The aim of this study was to assess the QoL of patients diagnosed with laryngeal cancer and qualified for surgical treatment before and after surgery with the use of EORTC QLQ-C30 and QLQ-H&N35 modules and to compare the results.

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METHODS

In this study, 54 patients hospitalized in the Otolaryngology, Head and Neck Surgery Department of the Medical University of Wroclaw who were diagnosed with laryngeal cancer in T3–T4 stages and all laryngeal locations qualified for surgical treatment were asked to fill out the paper version of the EORTC questionnaire, translated into the Polish language and validated, one day before surgery. It consists of a general module for patients diagnosed with cancer and a specific one for patients with head and neck cancer. The EORTC questionnaire was developed by Bjordal et al. [6] in 1994. The questionnaire consists of 37 items concerning many aspects like disease-related symptoms, social function, and sexuality. After a few years, a 3.0 version of the EORTC QLQ C-30 questionnaire was developed. The validity and reliability of both QLQ-C30 and H&N35 module was confirmed on a large group of patients from many different countries [7, 8]. Version 3.0 of the EORTC QLQ-C30 module contains 30 questions, and the H&N35 module contains 35 questions. Raw data collected from the questionnaire are calculated into the global, functional, and symptomatic scales according to the instructions provided in the scoring manual [9]. The questionnaire was well accepted and sensitive to changes during a study year. Many symptoms, such as problems with taste, swallowing difficulty, hoarse voice, and sore mouth, showed great variability [10].

From one to five years after surgery, correspondence with blank questionnaires was sent to all the patients with a request to fill out the questionnaires again or to send information about the possible death of the patients. All the data was collected and calculated with the instructions provided in the scoring manual for EORTC questionnaires. The calculated data created three types of scales. The data from the QLQ-C30 module was calculated into the global health status scale, five functional scales, and symptomatic scales. Functional scales are physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning. Symptomatic scales are fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. Further QLQ-H&N35 module data was calculated only into symptomatic scales like pain, swallowing, sensory problems, speech problems, trouble with social eating, trouble with social contact, less sexuality, teeth, opening mouth, dry mouth, sticky saliva, coughing, ill feeling, pain killers, and nutritional supplements. All the data calculated into scales creates a score of 0–100. For global health status, a higher score represents high QoL; in functional scales, a higher score represents a high and healthy level of functioning, whereas a higher score in symptomatic scales represents a high level of symptomatology and problems. The approval of ethical review board was obtained before the beginning of the study.

A statistical analysis of the obtained results was then performed using STATISTICA v. 12 (StatSoft, Tulsa, OK, USA). Statistical characteristics of variables are presented as arithmetical mean ± standard deviation, median, and interquartile range. Statistical characteristics of discrete variables are presented as number and frequency distribution. In the statistical analysis, the Wilcoxon matched pairs test was used for quantitative variables. Survival time was estimated using the Kaplan–Meier method.

RESULTS

The study sample characteristics are summarized in Table 1. Fifty-four patients 46–88 years old, including 50 males and four females, filled out the questionnaire before surgery. From one to five years after surgery, information from 31 patients was received. Twenty-one patients were reported dead. Ten patients filled out the questionnaire and sent it back to the clinic. Twenty-three patients did not answer the request.

Table 1. Age (years) of the respondents

Mean (SD)	60.5 (8.3)
Median (IQR)	59.0 (55.2–65.7)
Min–Max.	46–88

IQR – interquartile range; SD – standard deviation

Table 2. Sex of the respondents

Male	50 (92.6%)
Female	4 (7.4%)
Replies to the questionnaire	10 (18.5%)
Death	21 (38.9%)
No answer	23 (42.6%)

Scores for all scales compared before and after treatment are presented in Table 4. After surgery, the global QoL in patients with laryngeal cancer improved, but the difference was not statistically significant (54.2 *vs.* 50, p > 0.05) (Figure 1). The only statistically significant difference

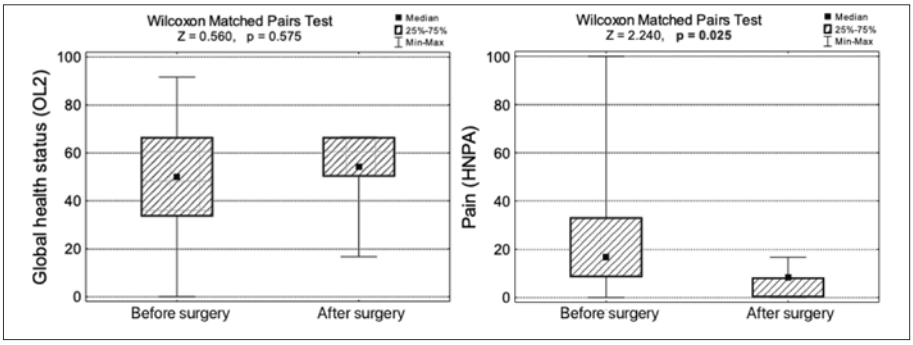

Figure 1. Comparison of the global health status before and after surgery and the result of the Wilcoxon matched pairs test

Table 3. Comparison of the QoL before and after surgery

Parameter	Before treatment		After treatment		p
	Median	IQR	Median	IQR	
Global health status/QoL	50	33.3–66.7	54.2	50–66.7	0.575
Physical functioning	80	66.7–93.3	73.3	66.7–86.7	0.161
Role functioning	83.3	66.7–100	83.3	66.7–100	0.345
Emotional functioning	66.7	50–75	70.8	58.3–91.7	0.308
Cognitive functioning	83.3	66.7–100	75	66.7–100	1.000
Social functioning	83.3	66.7–100	75	66.7–100	0.441
Fatigue	33.3	22.2–50	38.9	33.3–44.4	0.484
Nausea and vomiting	0	0–16.7	0	0–0	0.787
Pain	33.3	0–50	33.3	33.3–50	0.123
Dyspnoea	33.3	0–33.3	33.3	33.3–33.3	0.753
Insomnia	33.3	33.3–66.7	33.3	33.3–66.7	0.123
Appetite loss	0	0–33.3	0	0–33.3	0.345
Constipation	0	0–33.3	33.3	33.3–33.3	0.715
Diarrhoea	0	0–0	0	0–33.3	-
Financial difficulties	33.3	0–66.7	33.3	0–100	0.787
Pain	16.7	8.3–33.3	8.3	0–8.3	0.025
Swallowing	16.7	0–33.3	0	0–0	0.091
Senses problems	33.3	0–50	0	0–100	0.500
Speech problems	44.4	22.2–55.6	44.4	22.2–66.7	0.834
Trouble with social eating	8.3	0–33.3	0	0–0	0.176
Trouble with social contact	13.3	0–33.3	3.3	0–40	0.779
Less sexuality	33.3	16.7–50	33.3	0–50	0.343
Teeth	33.3	0–66.7	33.3	0–33.3	0.273
Opening mouth	0	0–0	0	0–0	-
Dry mouth	33.3	0–33.3	33.3	0–33.3	0.068
Sticky saliva	33.3	0–66.7	33.3	33.3–66.7	0.893
Coughing	33.3	33.3–66.7	33.3	33.3–66.7	0.295
Felt ill	33.3	0–66.7	33.3	0–66.7	0.447
Pain killers	0	0–100	50	0–100	1.000
Nutritional supplements	100	0–100	100	100–100	1.000
Feeding tube	100	0–100	100	0–100	1.000
Weight loss	0	0–100	100	0–100	-
Weight gain	100	100–100	100	0–100	0.593

IQR – interquartile range

occurred in the symptomatic scale for pain. Pain created more problems before surgery than after surgery (16.7 *vs*. 8.3; $p < 0.05$). Swallowing created more problems before surgery than after surgery. The p-value in this variable was on the border of statistical significance (16.7 *vs*. 0; $p = 0.091$).

The level of functioning mostly decreased after surgery beside emotional functioning and role functioning, but the changes were not statistically significant. The PF score was 80 before surgery and decreased to 73.3 after surgery. A similar situation was observed in CF, where the score decreased from 83.3 to 75, and SF, where the score also decreased from 83.3 to 75. The EF score increased from 66.7 to 70.8. RF kept the same level before and after surgery (83.3). All the aforementioned results are presented in Figure 2.

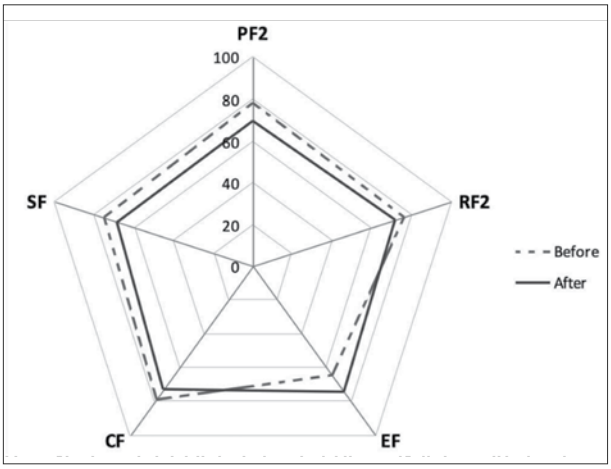

Figure 2. Comparison of the functional scales before and after surgery

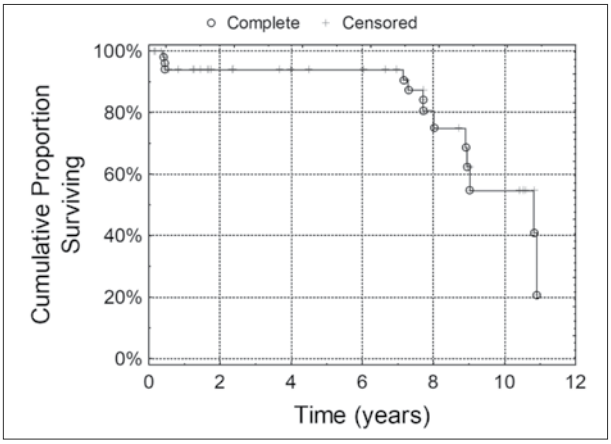

Figure 3. Kaplan–Meier's survival curve in the sample group

The length of survival after surgery is presented in Figure 3. In this study group, 90.6% of patients survived five years after surgery. The survival function was 8.01 for the 25th percentile, 9.63 for the 50th percentile, and 10.81 for the 75th percentile.

DISCUSSION

Because of its location and functional importance, the larynx plays a critical role in the maintenance of such cardinal physiological functions as phonation, regulation of respiratory airflow, and airway protection. Laryngeal cancer can have effects on laryngeal function, and the impact of treatment on function has to be carefully weighed against its oncological benefit. Still, in some cases, the only treatment is a total laryngectomy. Lately, the QoL reported by patients regardless of correlation with clinical parameters of health has paramount importance in treatment management. Comparison of the QoL in these patients might give better insight into patients' expectations and benefits from choosing the treatment method [11].

In this study, we compared the QoL in patients with laryngeal cancer qualified for surgical treatment before and

Table 4. Comparison of changes in functional scales after surgery in the literature and in our study

Scale	Singer et al. [12]		Zatoński and Kolator	
	Changes after surgery	p	Changes after surgery	p
PF2	deterioration	< 0.01	deterioration	0.16
RF2	deterioration	< 0.01	same level	0.34
SF	deterioration	0.02	deterioration	0.30
CF	deterioration	0.59	deterioration	1.0
EF	improvement	0.37	improvement	0.44

PF2 – physical functioning; RF2 – role functioning; SF – social functioning; CF – cognitive functioning; EF – emotional functioning

a few years after surgery. The results of our analysis show that the pain level after surgery is lower than before. Also, swallowing improved after surgery, but this change is on the border of statistical significance. A study by Singer et al. [12] was the only one found in the PubMed database that covers the same topic and uses the same questionnaire as the method of QoL assessment. The results of the multicenter prospective cohort study show that the QoL domains that improved were global QoL, coughing, and weight.

In our study, the changes in functional scales before and after surgery were statistically insignificant, but they found confirmation in the literature where the level of the same scales changed almost in the same way, and some of these changes were statistically significant.

A comparison is presented in Table 5 [12].

CONCLUSION

. Though our results are not valid to be generalized due to the number of the obtained follow-up questionnaires being smaller than the initial number of participants, it can, however, be assumed with a certain degree of probability that the QoL in patients after surgery improved in the domain of pain, taking into consideration our statistical analysis and discussed literature.

2. For all the domains, the result in the swallowing scale improved after surgery, and dry mouth remained on the same level. Changes in these scales were on the border of statistical significance.
3. In this study group, 90.6% of respondents survived five years after surgery.
4. Further research should be conducted on a larger group of patients. There are many studies about the QoL reported by patients with laryngeal cancer researching the relationship with the type of treatment method of surgery and other variables. All of these studies present statistically significant results. Because of different study protocols, it is hard to construct a global laryngeal cancer treatment algorithm that takes into account not only oncological benefit and treatment results but also patient-reported QoL. There is a need to conduct a bigger multicenter study based on the same examination and data sampling protocol. The obtained results could become a standard for care and proposed treatment choice.

The present study has certain limitations. Assessment of the QoL in patients with laryngeal cancer is a prospective study which has been continued after surgery to follow up potential changes in the QoL. We showed the results of 54 patients who agreed to fill out the QoL questionnaire. Because of the character of the study group and the method of sampling, the number of follow-up questionnaires we obtained was smaller than the initial number of participants. Although the questionnaire was sent to all the participants of this study after surgery, due to the death of some participants or the lack of willingness to re-fill the questionnaire, the response level in this study was low.

Conflict of interest: None declared.

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Квалитет живота болесника са раком ларинкса пре и после операције

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САЖЕТАК

Увод/Циљ Болесници са раком ларинкса хоспитализовани на Одељењу за оториноларингологију и хирургију главе и врата, квалификовани за хируршки третман, попуњавали су Упитник о процени квалитета живота пре и после операције.

Методе Педесет четири болесника са раком ларинкса у стадијумима T3 и T4 која су квалификована за потпуну ларингектомију замољена су да попуне модуле *EORTC KLK*-30 и *H&N*30 пре и неколико година после хируршког третмана.

Резултати Квалитет живота хоспитализованих болесника порастао је после операције. Ниво бола после хируршке интервенције се смањио и био је статистички значајан ($p = 0,025$). У студијској групи је 90,6% болесника преживело пет година после операције.

Закључак Квалитет живота болесника са раком ларинкса побољшан је у домену бола. Даље истраживање треба спровести на већој групи болесника. Будући резултати могу пружити користан материјал за анализу у вези са користима за болеснике и могу бити релевантни за одлуку да се пристане на предложени третман и избор његовог типа.

Кључне речи: квалитет живота; рак грла; праћење; ларингектомија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Chromatin textural parameters of blood neutrophils are associated with stress levels in patients with recurrent depressive disorder

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SUMMARY

Introduction/Objective During the past 20 years, there have been numerous attempts to design and apply a simple, affordable blood analysis tool for diagnostic and prognostic purposes in psychiatry. In this article we demonstrate that some mathematical parameters of chromatin organization and distribution in blood neutrophil granulocytes are related to stress levels in patients diagnosed with recurrent depressive disorder (RDD).

Methods The study was performed on 50 RDD participants who were asked to complete Depression, Anxiety and Stress Scales (DASS-21). Peripheral blood samples were obtained from all the participants, smeared on glass slides and stained using a modification of Giemsa method. A total of 500 representative chromatin structures (10 per patient) of neutrophil granulocytes were evaluated using textural analysis with the application of gray level co-occurrence matrix (GLCM) method. Parameters such as angular second moment (indicator of textural uniformity), inverse difference moment (textural homogeneity), and textural sum variance were calculated.

Results The results indicate that there is a statistically highly significant correlation ($p < 0.01$) between certain chromatin GLCM parameters such as inverse difference moment, and DASS-21 stress score. There was also a significant difference ($p < 0.05$) in some chromatin GLCM parameters in patients diagnosed with RDD with psychotic features, when compared to the ones without psychosis.

Conclusion These findings suggest that in the future, chromatin GLCM features might have a certain predictive value for some clinical features of recurrent depressive disorder.

Keywords: nucleus; structure; anxiety; depression

INTRODUCTION

In recent years, in the rapidly growing field of neurosciences, there have been many attempts to develop an exact, objective, and affordable image analysis method that would be applied in clinical practice as a supplement to the conventional diagnostic protocols. Many new mathematical algorithms have been proposed, often with limited results and impact. One of the techniques that are today being frequently considered in neurology and biology studies includes the analysis of texture [1, 2]. Textural analysis can be used to quantify structural features such as homogeneity and uniformity [2, 3].

There are many ways to assess texture of a biophysical system. Some of the methods are based on higher mathematics and second order statistical calculations. One of these frequently used textural algorithms include the Gray level co-occurrence matrix (GLCM). The parameters of GLCM method may have certain value in medical image analysis due to their potential ability to detect structural alterations in cells and tissues [4].

Textural parameters of tissue architecture were shown to be a potentially important addition to conventional cell biology and histology methods [5, 6]. For example, in a study published in 2009, Shamir and associates demonstrated that these features, when calculated on muscle tissue in an animal experimental model, can be a possible indicator of structural deterioration during physiological aging. In collagen morphology, textural indicators also exhibit a differentiating power to some extent [7]. Some textural parameters of spleen germinal center tissue might be associated with some physiological parameters such as humoral immune response to a foreign antigen [8].

Our recent studies have indicated that changes in chromatin structure in some cells can also be detected and evaluated using GLCM features. Our work on spleen lymphocytes in germinal centers indicated that some parameters are correlated with nuclear shape [9].

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Nuclear textural parameters of progenitor cells in spleen also exhibited statistically significant changes during aging [10]. Also, some of these determinants were proven to be very useful in discriminating two different lymphocyte populations in thymus [11]. Finally, in 2016, we indicated that textural features of blood lymphocyte chromatin may be important indicators of structural changes induced by oxidopamine [12].

In this study we show that some chromatin GLCM parameters of peripheral blood neutrophil granulocytes are associated with self-reported stress levels in patients diagnosed with recurrent depressive disorder. To our knowledge, this is the first work to show that GLCM features calculated under these conditions correlate to a psychological parameter of stress. Also, this is the first study to apply GLCM analysis in peripheral blood cells on a sample of psychiatric patients with recurrent depression.

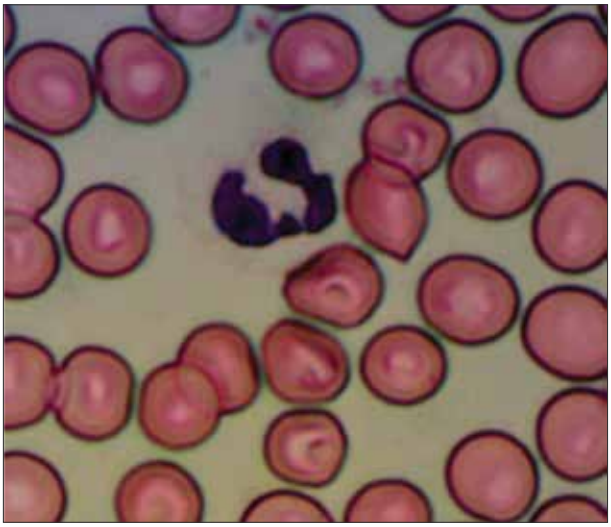

Figure 1. Giemsa-stained nucleus of a neutrophil granulocyte

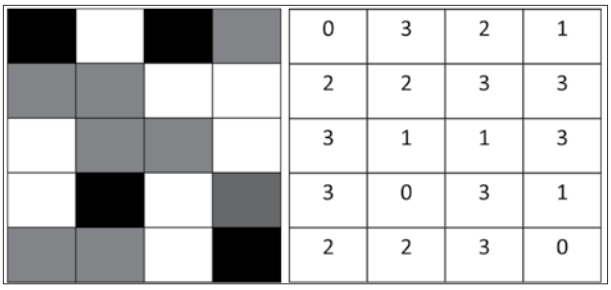

Figure 2. An example of gray level designations (for a low-quality micrograph) used for later formation of gray level co-occurrence matrix

METHODS

The study was performed on 50 patients (14 males, 36 females, average age 53.9 ± 6.4 years) at Laza Lazarević University Clinic for Mental Disorders, Belgrade, Serbia. All the patients had been diagnosed with recurrent depressive disorder (F33 diagnosis code according to International classification of diseases ICD-10). Exclusion criteria were as follows: comorbid psychotic or other serious psychiatric disorder, substance abuse, serious non-psychiatric disorders that might have impacted the final results (i.e. immunological illnesses, blood cell disorders, endocrine diseases, etc.).

All the participants were asked to complete a questionnaire that included Depression, Anxiety and Stress Scales (DASS-21) [13]. This instrument is made up of 21 self-report items that need to be rated on a Likert scale (four point). The items reflect emotional symptoms and experiences over the last seven days. Each of the three scales (for depression, anxiety and stress, respectively) consists of three items. The depression scale evaluates symptoms such as anhedonia, loss of self-esteem, and hopelessness. The anxiety scale refers to the symptoms related to fear, panic, worry, and autonomic system dysfunction. The stress scale covers items on relaxation difficulties, agitation, tendency to overreact, etc.

A sample of peripheral blood was obtained from all the participants and smeared on glass slides. The smears were fixated in methanol and stained using Giemsa method (Figure 1). For the details regarding the Giemsa technique, the reader is referred to previously published works of other authors [14, 15]. This method has also been successfully applied for textural analysis of chromatin in spleen follicular cells in our recent research [16]. The study protocol regarding DASS-21 administration, blood smear preparation and staining was a part of a wider research for a PhD thesis which was approved by the ethics panel of the host institution. Informed signed consent for the participation in the PhD thesis research was obtained from all the patients. The research was conducted in accordance with the Helsinki Declaration as revised in 1989 and conformed to the legal standards regarding research in medicine.

Nuclear structures of Giemsa-stained neutrophil granulocytes (10 cells per patient) were visualized using Pro-Micro Scan DEM 200 instrument (Oplenic Optronics, Hangzhou, CN) mounted on Olympus BX41 microscope (Olympus Corporation). Digital micrographs were created and saved in JPEG format. Dimensions of the micrographs were 1600 × 1200 pixels (width 1600, height 1200), both horizontal and vertical resolutions were 96 dpi, and bit depth equaled 24. Regions of interest of neutrophil nuclei were created, after which textural GLCM analysis of regions of interest was performed.

Parameters of image texture were calculated using MaZda software, previously developed for COST B11 and B21 European projects by a researcher from the Institute of Electronics, Technical University of Lodz [17, 18, 19]. Textural parameters were calculated based on Gray level co-occurrence matrix (GLCM) where second order statistical analysis is performed on resolution unit pairs and their gray values. Before GLCM is constructed, each resolution unit is assigned a value based on its gray intensity. The example of such assignment on a simpler micrograph is shown on Figure 2. Our micrographs had much higher number of possible gray intensity values compared to the one in the figure.

Inverse difference moment (IDM) as a measure of textural homogeneity was calculated based on the following formula:

$$IDM = \sum_i \sum_j \frac{1}{1+(i-j)^2} p(i,j)$$

where 'i' and 'j' are the values of neighbor and reference pixels in GLCM. Details on GLCM creation and features can be found in various previous works [2, 20].

Apart from IDM, we also measured the texture angular second moment (ASM). This is an indirect parameter of textural uniformity and can be determined as:

$$ASM = \sum_i \sum_j \{p(i,j)\}^2$$

Sum variance (SVAR) of the co-occurrence matrix was calculated as:

$$SVAR = \sum_{i=2} \left[i - \sum_{i=2} i p_{x-y}(i) \right]^2$$

This parameter is related to local variations of the textural distribution in a micrograph.

All GLCM parameters were calculated on regions of interest (objects) directly from the micrograph which was previously converted to 8-bit gray scale format. No cropping or other modifications were performed.

As the addition to the textural measurements, we also calculated fractal dimension (FD) of the neutrophil chromatin structure. FD was determined on binarized nuclear images, using the FracLac plugin for ImageJ (A. Karperien, Version 2.5, Release 1e, Charles Sturt University, Australia) [21]. During the analysis, a special box-counting method is performed, the structure is covered by a number of boxes (N) on different scales (ε), after which FracLac forms a logarithmic graph (Figure 3) and calculates FD from the slope of the regression line:

FD = regression slope [ln(N)/ ln (ε)]

FD was previously used on numerous occasions in cell biology in order to detect small structural alterations [16, 22]. Although not directly related to ASM or IDM, it may provide additional information on the nature and causes of their potential change.

RESULTS

Mean score for the depression and anxiety scales of DASS-21 were 10.9 ± 5.3 and 9.2 ± 4.4, respectively. The average value of stress score in DASS-21 was 11.7 ± 5.3. The mean value of IDM in neutrophil granulocytes was 0.79 ± 0.04. The average textural ASM of chromatin structure was 0.041 ± 0.021, and the mean value of nuclear FD was 1.52 ± 0.11. Mean SVAR of the GLCM equaled 73.9 ± 21.3.

There was a statistically highly significant ($p < 0.01$) correlation between neutrophil chromatin IDM and the score of the stress scale within DASS-21 (Figure 4). The correlation was negative, meaning that as the IDM increased, the stress level decreased, and vice versa. In Figure 4, plotted values of chromatin IDM and stress levels are shown. It was concluded that the age of the patients was not a contributing factor to this correlation. The relationship between stress score and IDM suggests that chromatin

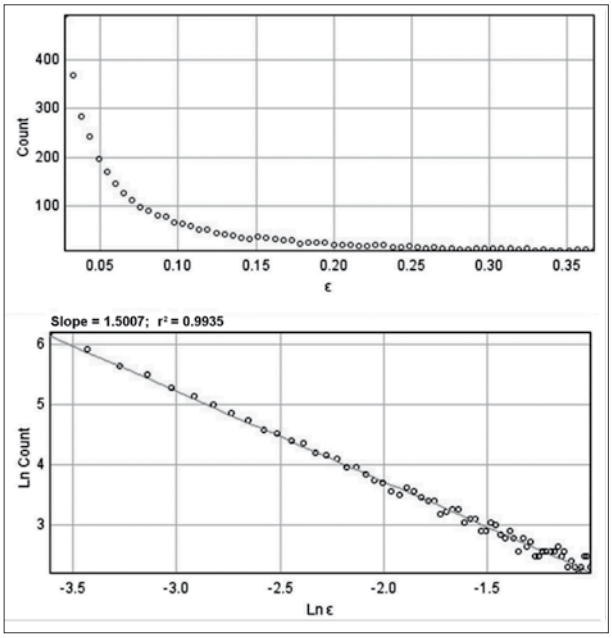

Figure 3. As an addition to this study, fractal analysis was performed using the box-counting method; in this method, the structure is covered by a number of boxes (N) on different scales (ε), after which the software forms a logarithmic graph and calculates fractal dimension from the slope of the regression line

IDM of blood neutrophils is a potentially good indicator of psychological distress in patients diagnosed with recurrent depressive disorder. No such correlation ($p > 0.05$) was observed between the values of chromatin IDM and the result of DASS-21 depression scale. Neither chromatin FD nor the values of chromatin ASM and SVAR in blood neutrophils were related to the scores of the DASS-21 scales. There was a much weaker negative relationship between IDM and DASS-21 anxiety score ($p < 0.05$, Figure 5).

Patients with psychotic features (n = 10) had significantly higher ($p < 0.01$) values of chromatin SVAR, compared to the patients without psychosis (n = 40). The values of SVAR equaled 92.7 ± 17.9 and 69.2 ± 19.5, respectively. ASM, IDM, and FD did not significantly differ between these two subgroups.

DISCUSSION

In this study in patients diagnosed with recurrent depressive disorder, we investigated the potential relationship between mathematical parameters of chromatin organization in peripheral blood neutrophils, and determinants of depression, anxiety, and stress. The main finding is the detected correlation between chromatin textural IDM and self-reported stress levels using the DASS scale. Other mathematical parameters were not significantly related to the scores of DASS subscales. These results imply that GLCM inversed difference moment of chromatin is potentially a valuable indicator of stress, and that it may in the future be used as an integral part of a biosensing system in psychology, psychiatry, and related disciplines.

Recurrent depressive disorder is one of the most common mental disorders seen in contemporary psychiatry

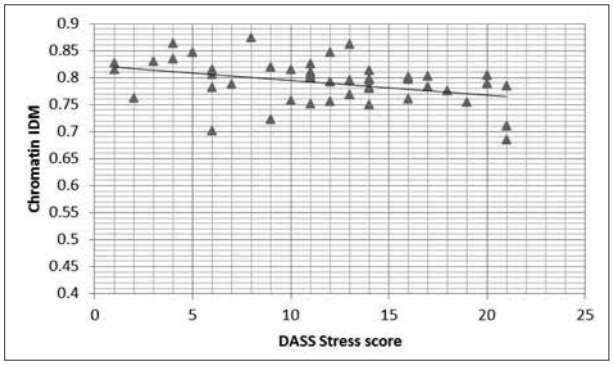

Figure 4. There was a statistically highly significant ($p < 0.01$) positive correlation between chromatin inverse difference moment (IDM) and Depression, Anxiety and Stress Scales (DASS-21) stress scores

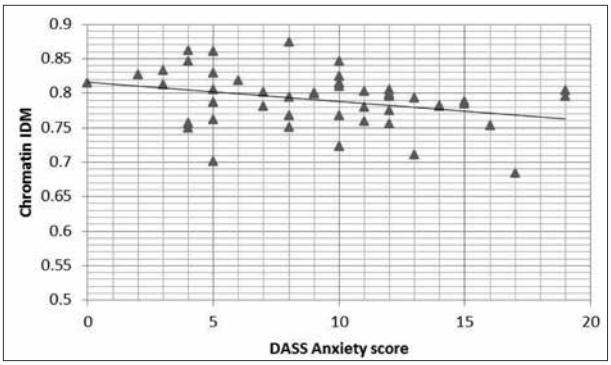

Figure 5. Plotted values of chromatin inverse difference moment (IDM) and Depression, Anxiety and Stress Scales (DASS-21) anxiety score; weak but significant correlation ($p < 0.05$) was detected

practice. The exact cause of depression has long been debated in medical research and today there are numerous theories trying to explain its pathogenesis and associated molecular mechanisms. One of such theories focuses on the possible impact of immune system and inflammation in the development of unipolar depression [23, 24]. Various cytokines and other immune-related mediators have been mentioned as potential contributors to depressive mood. Function of both neutrophils and lymphocytes may be changed during this disorder; however, the exact nature of these changes remains unclear.

Depression is closely related to stress, and the major finding of our study implies that the "stress component" of RDD is related to changes in neutrophil chromatin morphology. This may be due to several reasons. First, it is possible that cortisol, a well-known stress hormone, associated with "fight or flight" responses, may be involved in the manifestation of these chromatin changes. Depressed patients often have increased levels of cortisol, and this hormone when chronically increased might be a significant factor in depression development [25]. On the other hand, glucocorticoids exhibit significant genomic effects in neutrophil granulocytes [26]. Glucocorticoids may cause significant changes in expression of numerous genes, such as the ones for inflammatory cytokines, glutamine synthetase and other proteins. The magnitude of these changes may be similar to the ones seen in other leucocyte populations [26].

Second, it is possible that epinephrine and norepinephrine also significantly influence the chromatin organization of blood neutrophils. Kim et al. [27] showed that chronic catecholamine stress induced by prolonged delivery of epinephrine may significantly change the level of neutrophil trafficking. In some cell populations, epinephrine may substantially influence inflammation-related gene expression [28]. Changes in gene expression, if of sufficient magnitude, may alter the patterns associated with higher levels of DNA/chromatin organization, which can manifest itself as change in texture during conventional microscopy.

Finally, as correlation is not necessarily the proof of causality, we could speculate that the patients with specific patterns of neutrophil chromatin may have different self-perceived stress levels due to some specific properties of neutrophil function. Neutrophils are secretory active cells which produce a variety of chemical mediators. Some of those mediators (i.e. specific interleukins) might be responsible for the subjective feeling of distress. It can be assumed that neutrophils that are more (or less) secretory active will differ from others in terms of their chromatin organization. This may be related to altered euchromatin/heterochromatin ratio, different chromatin distribution within the nucleus, different interaction between chromatin and nuclear envelope. All these factors might be associated with changes in the values of overall chromatin homogeneity.

According to our opinion, the major contribution of our present study is not the investigation of a specific physiological mechanism of stress and depression, but the fact that the GLCM IDM as a mathematical parameter has some prognostic value in assessing the stress levels in depressed patients. To our knowledge, to this date no parameter of blood neutrophils exists that would be able to serve as an indicator of stress in RDD. In fact, this is probably one of the first works to demonstrate the potential clinical value of neutrophil chromatin mathematical analysis, not only in RDD, but in psychiatry in general. In the future, it would be interesting to see if chromatin IDM is capable of predicting the outcomes of RDD therapy, or to correlate it with other biological tests used in contemporary psychiatry research.

Also, the potential value of our results reflects in the fact that GLCM analysis of blood neutrophils is an exact, objective, and relatively affordable method which does not require significant time and financial resources. Conventional histology and pathology analysis often rely on the subjective opinion of the professional on the appearance of cell and subcellular components. For example, to a pathologist, a nucleus may "appear" more or less disorderly in its structure and texture, but using the conventional means, so far it hasn't been possible to assign quantification to this evaluation. Mathematical textural analysis overcomes this issue and applies a precise and objective estimate of structural features which can be performed by almost any medical or biological professional. This opens up numerous future possibilities regarding a design of modern easy-to-use biosensors in psychophysiology and psychiatry.

Our study had certain limitations that need to be pointed out. First, we opted for Giemsa staining method, which is today commonly used in cytogenetics. This method targets the phosphate groups of DNA, and especially the areas of genome rich with adenine–thymine segments. There are however numerous other staining techniques that are able to adequately assess the structure of a nucleus. In the future, we recommend additional experiments to be performed using DNA-specific Feulgen method, as used in our previous study on adrenal gland tissue [29]. In terms of some aspects of interaction with the DNA molecule, this method might be superior to Giemsa, and it would certainly be interesting to see results on textural analysis after administration of this stain. Also, our study measured the levels of stress in depressed patients using DASS-21 psychiatric scale, which is today frequently used to quantify subjective distress in both patients and healthy subjects; there are, however, numerous other means to evaluate stress in clinical conditions. Future studies would also need to include various different physiological and psychiatric methods for stress assessment and try to test if the mathematical analysis is still a good indicator of stress under the new conditions.

CONCLUSION

Our study indicates that in patients diagnosed with recurrent depressive disorder, some textural features of blood neutrophil granulocytes are associated with the levels of subjective distress. To our knowledge, this is the first study to perform textural analysis of blood leucocyte chromatin in this experimental setting. The results suggest potential applicability of mathematical analysis of blood cell chromatin in future psychiatry and psychophysiology research.

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The research protocol regarding the administration of the DASS-21 and obtaining/staining the patients' blood was done as a part of PhD thesis work of the co-author Draga Dimitrijević.

Conflict of interest: None declared.

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Текстурални параметри хроматина неутрофилних гранулоцита крви су асоцирани са степеном стреса код болесника са рекурентним депресивним поремећајем

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САЖЕТАК

Увод/Циљ Током протеклих 20 година постојали су бројни покушаји дизајнирања и примене једноставног, приступачног метода за анализу крви за дијагностичке и прогностичке потребе у психијатрији.

У овом раду показујемо да су неки математички параметри организовања и дистрибуције хроматина у неутрофилним гранулоцитима крви повезани са нивоима стреса код болесника са дијагнозом рекурентног депресивног поремећаја.

Методе Студија је спроведена на 50 болесника са дијагнозом рекурентног депресивног поремећаја, од којих је затражено да попуне упитник за депресију, анксиозност и стрес (*DASS*-21). Узорци периферне крви добијени су од свих учесника, направљени су размази на предметним стаклима и обојени коришћењем модификације методе по Гимзи. Укупно 500 репрезентативних хроматинских структура (10 по болеснику) неутрофила анализирано је коришћењем текстуралне анализе, односно математичког алгоритма *GLCM* (*gray level co-occurrence matrix*). Израчунати су параметри попут ангуларног другог момента (индикатор текстуралне униформности), инверзног момента разлике (текстурална хомогеност) и текстуралне суме варијансе.

Резултати Резултати показују да постоји статистички значајна корелација ($p < 0,01$) између одређених *GLCM* параметара хроматина, као што је инверзни моменат разлике, и *DASS*-21 скора за стрес. Постојала је и значајна разлика ($p < 0,05$) у неким *GLCM* параметрима хроматина код болесника са дијагностификованим депресивним поремећајем са психотичким карактеристикама у поређењу са онима без психозе.

Закључак Ови налази указују на то да текстурална анализа хроматина крвних ћелија у будућности може имати одређену предиктивну вредност за неке клиничке особине рекурентног депресивног поремећаја.

Кључне речи: једро; структура; анксиозност; депресија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Physical birth outcomes in neonates prenatally exposed to buprenorphine – our first experiences

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SUMMARY
Introduction/Objective Buprenorphine appears generally similar to, and in some cases superior to, methadone in terms of maternal, fetal, and neonatal outcomes.
The objective of the study was to assess some physical birth outcomes in neonates prenatally exposed to buprenorphine.
Methods During a seven-year period, nine patients have been treated with buprenorphine during their pregnancy. All women underwent interview, clinical investigations, biochemical analysis, toxicological screening, viral markers for hepatitis B, C, HIV, with regular check-ups by an obstetrician and a psychiatrist. Newborn outcomes included: birth weight in grams, birth length in centimeters, physical anomalies, head/chest circumference in centimeters, Apgar score at 1 minute / 5 minutes, gestational age (weeks), newborn length of hospital stay in days, breast-feeding, the newborn's need for pharmacologic treatment after delivery.
Results The mean birth weight was 2,991.11 ± 37 g; birth length was 49.44 ± 2.29 cm; head circumference was 33.11 ± 0.78 cm; chest circumference was 32.33 ± 1 cm; first minute Apgar score was 8.22, fifth minute 9.22; age at delivery was 38.77 ± 1.09 weeks; hospitalization after delivery 4.44 ± 1.13 days. None of the newborns had physical anomalies. Six of the newborns were breastfed.
Conclusion Buprenorphine is a safe and important part of a complete comprehensive treatment approach in pregnant women with opioid use disorder. Buprenorphine treatment of maternal opioid use disorder indicated a low risk of preterm birth, normal birth weight and length, head and chest circumference, Apgar score, short hospitalization after delivery.
Keywords: buprenorphine; pregnancy; neonates; physical birth outcomes

INTRODUCTION

Substance use disorder among pregnant women continues to be a major public health concern, posing risk to the child's development, and imposing socioeconomic burdens on society by increasing needs for medical and social services [1]. Given the increasing prevalence of use of opioids by pregnant women, and the potentially serious maternal, fetal, and neonatal risks attendant to such use, the provision of effective treatment for this population should be a public health priority [2].

From 2002 to 2013, the largest increase in heroin use was among women. The rate of opioid use during pregnancy is approximately 5.6 per 1,000 live births, with one study reporting greater than 85% of pregnancies in women with opioid use disorder (OUD) were unintended. Opioid agonist therapy is the first-line recommendation for pregnant women with OUDs. The goals of treatment are to manage withdrawal, reduce cravings, and provide opioid blockade (preventing euphoria from illicit use). The goals of opioid agonist therapy in pregnancy are to prevent illicit opioid use, which can increase the risk of fetal growth restriction, *abruptio placentae*, fetal death, preterm labor, and intrauterine passage of meconium. Opioid agonist therapy has been shown to increase adherence to prenatal care, reduce illicit drug use, reduce infection exposure secondary to intravenous drug use, such as HIV, HCV, improve maternal nutrition, and improve infant birth weight [3].

The accepted treatment for OUD during pregnancy is long-acting opioid agonist medication-assisted treatment, such as methadone or buprenorphine, within the context of a comprehensive program of obstetric care and psychosocial interventions [4].

Buprenorphine appears generally similar to, and in some cases superior to, methadone in terms of maternal, fetal, and neonatal outcomes. Like methadone, prenatal buprenorphine exposure appears to be associated with a clinically significant neonatal abstinence syndrome (NAS) requiring pharmacological intervention in approximately half of the cases. However, results from the MOTHER study suggest that buprenorphine is associated with a less severe NAS than methadone. Generally positive outcomes for both mother and child following buprenorphine exposure in randomized controlled trials were achieved in the context

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of receiving flexible and adequate buprenorphine dosing during pregnancy and postpartum, and comprehensive treatment from a multi-disciplinary team. While the nature of science is to compare and contrast treatments in order to discover which treatment is better, the reality is that no one treatment will be maximally effective for all patients. Our collective commitment should be towards researching which treatment works best for which patients [2].

The objective of the paper was to assess physical birth outcomes in neonates prenatally exposed to buprenorphine (the way of delivery, sex, birth weight, birth length, physical anomalies, head/chest circumference, Apgar score, gestational age, newborn length of hospital stay in days, breast feeding, newborn-required pharmacologic treatment after delivery).

METHODS

The University Clinic of Toxicology, Mother Teresa Clinical Center, started the treatment of OUD with buprenorphine in our country for the first time on August 1, 2009. Patients with Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-IV) diagnosis of OUD were treated according to Clinical Guidelines for the Use of Buprenorphine in the Treatment of Opioid use Disorder –Treatment Improvement Protocol (TIP 40). Patients were treated with buprenorphine sublingual tablets. By January 31, 2017, a total of 235 patients have been receiving treatment of OUD with buprenorphine and 23 of them have been women. This number varies from one month to the next. During this period, nine patients were treated with buprenorphine throughout their pregnancy. All the women had previously started OUD treatment with buprenorphine. Besides previously made examinations, all of the women who became pregnant underwent additional investigations: interview, clinical investigations, biochemical analysis, toxicological screening, viral markers for hepatitis B, C, HIV, underwent regular check-ups by an obstetrician and a psychiatrist. All the patients underwent regular check-ups and were positive on buprenorphine in urine sample. On several occasions during pregnancy, one patient was positive on THC, and one patient was twice positive on benzodiazepines during pregnancy. All the patients during pregnancy were negative on opiates, methadone, cocaine, amphetamines, tramadol. They were also Hbs Ag, antiHCV, and HIV negative. All the mothers were receiving buprenorphine and there was no medication change. The women remained on their opioid maintenance therapy. Breastfeeding was encouraged.

Newborn outcomes included the following information: the way of delivery, sex (male/female), birth weight in grams, birth length in centimeters, physical anomalies, head/chest circumference in centimeters, Apgar score 1 minute / 5 minutes, gestational age (weeks), newborn length of hospital stay in days, breast feeding, the newborn's need for pharmacologic treatment after delivery.

The following instruments were used for testing: toxicological analyses in urine samples (fluorescence polarization immunoassay – FPIA); qualitative tests for buprenorphine and tramadol. All the tests were performed at the Institute of Forensic Medicine, University Clinic of Toxicology. The head circumference was measured above the ears equally on both sides and across the occipital font. The chest circumference was measured across the nipple line around the back of the newborn during exhalation. The circumference measurements and body length were taken with a measuring tape. Body weight was measured according to the Procedure for Weighing Infants/Children using a Digital Infant Scale. Gestational age assessment was according to Dubovitz–Ballard score.

Maternal characteristics included the following: age (years), education, which psychoactive substances were used before treatment, route of psychoactive substances administration before treatment, which pregnancy in a row, way of parturition, miscarriage, buprenorphine dose (mg) during pregnancy, time of treatment before pregnancy (months).

Inclusion criteria were as follows: all the pregnant women had a Diagnostic and Statistical Manual of Mental Disorders IV diagnosis of current OUD and maintenance treatment with buprenorphine, positive buprenorphine test, negative opiates, methadone, cocaine, amphetamines, and tramadol test.

Exclusion criteria were the following: patients who dropped out from the maintenance treatment with buprenorphine by their own volition; patients who did undergo regular check-ups, patients with negative buprenorphine test.

This treatment was carried out with the approval from the Ministry of Health (National Program for the Treatment of Patients with OUD) and from the Institutional Board of University Clinic of Toxicology. All the patients underwent this treatment with written consent.

Descriptive statistics was done with the statistical program SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

This study included nine pregnant women on treatment with buprenorphine for OUD. The mean age of these patients was 27.22 ± 2.58 years. Before treatment, five of the patients had heroin use disorder alone, three had polydrug use disorder, and one took methadone from the "black market." Two of them took opioid intravenously, but all were HbsAg, antiHCV, and HIV negative. It was the first pregnancy for all of them. Only one patient had spontaneous miscarriage after her first pregnancy, due to an unknown reason. Two patients had cesarean section. The mean dose of buprenorphine during pregnancy was 11.11 ± 4.37 mg. The lowest dose was 6 mg and the maximum dose was 16 mg. The average treatment duration with buprenorphine before pregnancy was 21.44 ± 9.72 months. In the end, in two of all the included patients, the dose was slowly reduced and they finished the treatment with buprenorphine.

Table 1. Maternal characteristics

Patient	Age (year)	Education	Use of PAS before treatment	Route of PAS administration before treatment	Pregnancy	Miscarriage	Buprenorphine dose (mg) during pregnancy (mg)	Time of treatment before pregnancy (months)
1	27	secondary	heroin	inhalation	First	none	16	18
2	25	secondary	heroin	inhalation	First	none	6	9
3	29	university degree	Methadone, benzodiazepines	*Per os*	First	none	10	24
4	31	university degree	Methadone, benzodiazepines, tramadol, heroin	Inhalation, *per os*	First	none	12	19
5	30	secondary	heroin	inhalation	First	none	16	11
6	29	secondary	methadone	intravenously	First	none	16	14
7	24	university degree	heroin	intravenously	First	one	6	28
8	25	university degree	Methadone, heroin, benzodiazepines	Inhalation, *per os*	First	none	12	36
9	25	secondary	heroin	inhalation	First	none	6	34

PAS – psychoactive substances

Table 2. Physical birth outcomes in neonates prenatally exposed to buprenorphine with serial number corresponding to the serial number of the mother in Table 1

Neonates	Way of delivery	sex	Mean birth weight (gm)	Mean length (cm)	Physical anomalies	Head/chest circumference (cm)	Mean Apgar score 1 min. / 5 min.	Age at delivery (weeks)	Days of hospitalization	Breast feeding	Treatment after delivery
1	spontaneous	f	2750	50	no	33/32	9/10	39	4	No	/
2	spontaneous	m	2950	51	no	33/32	8/9	38	5	Yes	/
3	spontaneous	f	3100	49	no	32/31	8/9	39	6	Yes	/
4	caesarean section	m	3350	51	no	34/33	9/10	40	5	No	/
5	spontaneous	f	2200	44	no	32/31	8/9	38	4	Yes	/
6	spontaneous	f	2830	48	no	34/32	8/9	38	3	Yes	/
7	cesarean section	m	3150	50	no	33/33	8/9	40	6	Yes	/
8	spontaneous	m	3150	51	no	33/33	8/9	38	3	No	Phenobarbitone 2 × 5 mg
9	spontaneous	m	3440	51	no	34/34	8/9	40	4	yes	/

Maternal characteristics are outlined in Table 1.

In Table 2, newborn outcomes are presented, with serial number corresponding to the serial number of the mother in Table 1. Most of the patients (n = 7) had spontaneous parturition, only two patients had cesarean section. Five of the newborns were boys. The mean birth weight was 2,991.11 ± 37 gm and the mean length was 49.44 ± 2.29 cm. None of the newborns had physical anomalies. The mean head circumference was 33.11 ± 0.78 cm and the mean chest circumference was 32.33 ± 1 cm. The mean Apgar score was 8.22 in the first minute, and 9.22 in the fifth minute after delivery. The mean age at delivery was 38.77 ± 1.09 weeks. The mean length of hospital stay was 4.44 ± 1.13 days. Six of the newborns underwent breastfeeding. One of the patients stopped with breastfeeding at her mother's suggestion, and the other two newborns were formula-fed. Phenobarbitone was prescribed in only one newborn as prevention from seizures, while the remaining eighth newborns received no treatment.

Physical birth outcomes in neonates prenatally exposed to buprenorphine are outlined in Table 2.

DISCUSSION

In 2009 for the first time in our country, University Clinic of Toxicology offered patients with OUD an alternative way of treatment with buprenorphine. By 2017, nine patients were treated with buprenorphine throughout their pregnancy. The American College of Obstetricians and Gynecologists has urged that buprenorphine be considered first-line treatment, but methadone is likely still the gold standard due to slightly higher adherence, more tightly controlled dosing, and insufficient evidence that buprenorphine is superior to methadone treatment [1].

As far as the dose of buprenorphine during pregnancy in this study, the mean dose of buprenorphine was 11.11 ± 4.37 mg. The lowest dose was 6 mg and the maximum dose was 16 mg. Similar results were shown in a study by Farid et al. [5], where buprenorphine doses used to maintain the pregnant woman were variable, with a mean dose range of 5.3–18.7 mg/day. In our study, the mean gestational age was 38.77 ± 1.09 weeks. Fourteen other non-randomized studies got similar results [2, 6].

This study reported no physical birth anomalies. Similar results were reported in MOTHER and PROMISE study [2].

In our work, the mean birth weight of neonates was 2,991.11 ± 37 g and the mean length was 49.44 ± 2.29 cm. The mean head circumference was 33.11 ± 0.78 cm and the mean chest circumference was 32.33 ± 1 cm. The mean Apgar score was 8.22 in the first minute, and 9.22 in the fifth minute after delivery. Similar studies that reported summary data suggest most neonates were full-term and within normal limits: birth weight (20 studies: 3,087.2 g), birth length (10 studies: 49.4 cm), and head circumference (nine studies: 34 cm) [2, 7]. Coulson et al. [8] in one retrospective cohort study conducted in a comprehensive, perinatal program in western North Carolina reported that differences in neonatal outcomes reached statistical significance for larger head circumference for buprenorphine doses, and for greater length with low to moderate dose buprenorphine versus high dose methadone. Similar results were reported in national registry studies from the Czech Republic and Norway, with 333 and 235 women, respectively, using opioid maintenance treatment during pregnancy [9].

Findings in one study by Nguyen et al. [6] showed neonatal outcomes (prenatally exposed to buprenorphine) within normal ranges for delivery and growth parameters. Moreover, mean birth weights have been mostly above 2.9 kg, with the lowest weight reported at 2.796 kg. Farid et al. [5] in their study reported that in most pregnancies birth weight, Apgar scores, head circumference, and body length were within normal ranges.

Methadone and buprenorphine are widely used to treat OUD. However, compared with methadone, buprenorphine is associated with shorter treatment duration, less medication needed to treat NAS, and shorter hospitalization for neonates [7, 10]. In our study, the mean length of hospital stay was 4.44 ± 1.13 days. Six of the newborns underwent breastfeeding. Phenobarbitone was prescribed in only one newborn as prevention from seizures, while the remaining eighth newborns received no treatment.

The present study has some limitations. The presented patients were the only ones on maintenance treatment with buprenorphine at the University Clinic of Toxicology; this study included only nine cases; a larger series of patients is necessary in order to reach conclusions on the association between pregnancy and OUD treatment with buprenorphine; more neonatal growth factors have to be observed.

CONCLUSION

Buprenorphine is a safe and important part of a complete comprehensive treatment approach for pregnant women with OUD. Buprenorphine treatment of maternal OUD indicated a low risk of preterm birth, normal birth weight and length, head and chest circumference.

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Физичке карактеристике новорођенчади пренатално изложене бупренорфину – наша прва искуства

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САЖЕТАК

Увод/Циљ Бупренорфин је уопштено сличан а у неким случајевима бољи од метадона за мајку, фетус и новорођенчад. Циљ рада је процена неких физичких карактеристика новорођенчади пренатално изложених бупренорфину.

Методе У периоду од седам година, девет жена са болешћу зависности од опиоида лечено је бупренорфином током трудноће. Све труднице су подвргнуте интервјуу, клиничким испитивањима, биохемијским анализама, токсиколошком скринингу, одређивању вирусних маркера за хепатитис Б, Ц, ХИВ и редовно су контролисане од стране акушера и психијатра. Прослеђени параметри код новорођенчади су порођајна тежина у грамима, порођајна дужина у центиметрима, физичке аномалије, обим главе/груди у центиметрима, Апгар скор 1 мин. / 5 мин., гестацијска старост (недеља), дужина болничког боравка у данима, дојење, фармаколошки третман после порођаја.

Резултати Просечна порођајна тежина је 2991,13 ± 37 *g*; просечна порођајна дужина је 49,44 ± 2,29 *cm*; обим главе 33,11 ± 0,78 *cm*; обим груди 32,33 ± 1 *cm*; Апгар скор 8,22/9,22; гестацијска старост 38,77 ± 1,09 седмица; дужина болничког боравка 4,44 ± 1,13 дана. Сва новорођенчад била су здрава, без конгениталних аномалија.

Закључак Бупренорфин је сигуран и важан део свеобухватног третмана код трудница са болешћу зависности од опиоида. Лечење бупренорфином резултирало је ниским ризиком од превременог порођаја, нормалном порођајном тежином и дужином, обимом главе и груди, нормалним вредностима Апгар скора, кратким хоспитализацијама после порођаја.

Кључне речи: бупренорфин; трудноћа; новорођенчад; физичке карактеристике после порођаја

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of combined antioxidant treatment on oxidative stress, muscle damage and sport performance in female basketball players

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SUMMARY

Introduction/Objective We determined the impact of antioxidant supplementation by GE132® on sports performance, oxidative stress markers, and muscle enzymes activities in professional female basketball players.

Methods Repetitive strength, explosive power, anaerobic endurance, and agility performance were measured before/after the 45-day supplementation period. The FORT (Free Oxygen Radicals Test) and FORD analysis (Free Oxygen Radical Defense) were assessed before/after basketball specific exercise bout, at the beginning/end of observational period. The grade of muscle damage was evaluated by aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH).

Results After supplementation period, significant difference was not recorded regarding the basic motor skills tests. Basketball specific exercise bout induced significant increase in FORT ($p < 0.05$) only at the beginning of supplementation period. Both FORT and FORD significantly decreased over the observational period ($p < 0.001$, $p < 0.01$, respectively). CK and LDH were remarkably lower at the end of observational period ($p < 0.05$), compared to the baseline.

Conclusion Exogenous supplementation with protective nutraceuticals such as those found in GE132®, could reduce acute/chronic oxidative stress and muscle damage, but had no effect on sport performance in basketball players.

Keywords: antioxidants, oxidative stress; FORT; FORD; basketball; muscle damage

INTRODUCTION

Moderate physical activity, associated with a balanced diet, provides numerous health benefits. However, exhaustive and/or intense training sessions are associated with increased production of reactive oxygen species (ROS) and might lead to oxidative stress (OS) in skeletal muscles, blood, and perhaps other tissues [1, 2]. An increasing body of evidence implicates OS in the pathogenesis of numerous diseases, including diabetes, certain cancers, and cardiovascular disease. Importantly, exercise-induced OS might be associated with fatigue, muscle damage, and increased recovery time, which can all affect exercise performance [3–6].

The body contains an antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as antioxidant enzymes and numerous non-enzymatic antioxidants, involved in the quenching or removal of free radicals [7]. Physical training may enhance the antioxidant defense system to offset the barrage of ROS generated during exercise [8, 9]. However, the body's natural antioxidant defense system might not be sufficient to counteract the increase in ROS production during high-intensity or prolonged intermittent aerobic or anaerobic exercise [10, 11]. Additionally, a large number of athletes failed to ingest sufficient quantities of fruits and vegetables, which also suggest suboptimal intake of various antioxidants.

Previous research has demonstrated that antioxidants obtained through antioxidant rich diet or supplementation can reduce lipid peroxidation [12–15] and muscle damage [13, 16–19], indicating reduced OS. Although isolated studies reported potential ergogenic properties of antioxidants [20, 21, 22], overall conclusion is that there is limited evidence that dietary supplementation with antioxidants improves human performance.

We investigated the effect of proprietary nutraceutical blend GE132® on OS, muscle damage and sport performance in female basketball players. This blend contains several components with various biological effects including antioxidant properties. Each capsule contains 100 mg of *Ganoderma lucidum* extract (20%), 130 mg of royal jelly (26%), 80 mg of resveratrol (16%),

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30 mg of shark protein complex (6%), 80 mg of green tea extract (16%), and 80 mg of rosehip extract (16%). It was shown that these components have many therapeutic effects including anticarcinogenic and antihypertensive effects, immunomodulatory effect, rheumatism alleviation, and hepatic disease prevention, health benefits related to gastrointestinal disorders, metabolic diseases, and allergies [23–26].

The efficacy of this blend has never been tested before. Because of the great interest in using antioxidant nutrients as a preventive and therapeutic tool in clinical medicine and in physical activity, the aim of the present study was to determine the effects of this dietary supplement on OS, muscle damage and sport performance in female basketball players during competitive half season. It was hypothesized that athletes would demonstrate lower OS and muscle damage in response to exercise and training after supplementation period.

METHODS

Subjects

Fourteen senior female basketball players, who play in the first league club Red Star, Belgrade, Serbia, participated in this study. Athletes gave written consent after explanation of the purpose, demands, and possible risks associated with the study. The protocol was in accordance with the Declaration of Helsinki for Research on Human Subjects and it was approved by the Ethical Committee of Sport Medicine Association of Serbia. All participants passed sports medical examination and were eligible for participation in competitive sport. None of the subjects reported any serious injury or disease six months prior to or during the study. All subjects were non-smokers and did not use oral contraceptives, anti-inflammatory drugs, or dietary supplements (i.e. antioxidants) one month before and during the study. Subjects were instructed to restrain themselves from making any drastic changes in the diet. All of them had regular menstrual cycles and none of them were in the menstrual phase at the time of blood sampling.

Study design and supplementation

The study was conducted during a competitive half season, over the 45-day period. During this period, athletes were engaged in a controlled training program, and participation in the study did not have any effect on previously determined training and competition schedule. Subjects completed two basketball specific exercise bouts, at the beginning and at the end of the observational period. Supplementation started after the first exercise bout and continued for 45 days. Before and after each exercise bout, capillary blood samples were collected for OS measurement. Venous blood samples were collected at the beginning and at the end of observational period for muscle enzyme analysis. In addition, all players performed basic motor skills tests at the beginning and at the end of the study, in order to evaluate strength, endurance, and agility as the most commonly used motor skills in basketball.

All subjects received antioxidant complex supplement, GE132®, during 45 days. Athletes were told to comply with supplementation protocol and to take two capsules daily, one before lunch and the other before dinner. Capsules were counted upon return of the capsule bottles to assess compliance.

Procedures and measurements

Baseline measurements: Prior to enrolling in the study, all subjects completed a body composition assessment, standard blood chemistry screening, medical history, and physical activity questionnaire. Anthropometric and body composition characteristics were determined by using Seca height measuring instrument (Seca GmbH, Hamburg, Germany) and Tanita scale BC-418MA (Tanita Corp., Tokyo, Japan).

Basketball-specific exercise bout: Each exercise bout consisted of a general warm up and stretching (approx.10 min), technical-tactical training (approx. 30 min), heavy training, including training of counterattacks and simulated full- or half-court basketball games (approx. 40 min), and finally a cool-down phase (approx. 10 min). Each subject served as self-control to eliminate any biological variability in the response to antioxidant supplementation. The exercises were carried out under the same conditions, in the same place and time of the day to avoid circadian variations.

Basic motor skills tests

Strength: In order to evaluate repetitive strength, players performed push-ups and sit-ups to failure. Push-ups were done by placing hands just wider than shoulders. Subjects were told to keep their elbows fairly close to body and point them back and not to flare them out to the sides. They lowered until their chests were just above the floor, paused for a split second, and then pressed themselves back up. In order to do sit-ups, subjects were told to raise the torso from a supine to a sitting position and then lie back down again without moving the legs. Knees were bent at an angle of 90°and arms were held crossed behind the neck during the test. Only correct repetitions were taken into account.

Explosive strength was assessed by Globus Ergo Tester Platform. Squat jump (SJ), countermovement jump (CMJ), vertical jump (VJ), left leg (LL) and right leg (RL) jumps values were obtained. The subjects stood on the contact platform connected to a digital timer that recorded the flight time and height of all jumps. The timer was triggered by the release of the player's feet from the platform, and stopped at the moment of touchdown. SJ was performed from a starting position of 90° knee angle without allowing any counter movement. The subjects were told to jump as high as they can without performing a countermovement. The hands were held on the hips during the jump, thus avoiding any arm swing. During the CMJ, subjects were in the position with knees slightly bent and moved into a semi-squat position before jumping. Subject's hands also remained on the

hips throughout CMJ. One leg jumps (RL and LL) were performed in the same way as the CMJ. The only difference was that subjects were jumping from and landing on the same leg. Considering the VJ, the similar technique was used like in the CMJ, but subjects were able to perform the arm swing during the jump. Each player performed three jumps and the highest values achieved were recorded.

Endurance: Anaerobic endurance, as an important aspect of basketball, was evaluated by 300-yard shuttle test. Marker cones and lines were placed 25 yards apart to indicate the sprint distance. Stopwatch was used to record results of the test. Athletes were told to run as fast as they can to the opposite 25-yard line, touch it with their foot, turn and run back to the start. This was repeated six times without stopping (covering 300 yards total). After a 5-minute rest, the test was repeated. The average values of the two 300-yard shuttles were recorded.

Agility: Agility performance of basketball players was assessed by agility t-test. Four cones were set in the court (5 yards = 4.57 m, 10 yards = 9.14 m), and the subjects started at cone A. When the times sounded off, the subjects sprinted to cone B and touched the base of the cone with their right hand. Then, they turned left and shuffled sideways to cone C, also touching its base, this time with their left hand. The subjects were then shuffling sideways to the right to cone D, touching the base with the right hand. At last they shuffled back to cone B, touching it with the left hand, and ran backwards to cone A. The stopwatch was stopped as they passed the cone A. The test was performed three times and average value of all three attempts was taken into account.

Oxidative stress and biochemical measurements: In order to evaluate OS status, approximately 15 minutes before and 15 minutes after each basketball specific exercise bout, capillary blood samples were collected for FORT (free oxygen radicals test) and FORD (free oxygen radical defense) measurements. The free radical analysis system FORM PLUS 3000 (Callegari S.P.A., Parma, Italy), incorporating a spectrophotometric device reader, was used to measure these parameters. Test kits used with this instrument are highly reliable, rapid, and user-friendly for the global evaluation of the oxidative status (radical-induced damage index and the total antioxidant capacity) in the body from capillary blood.

FORT assay provides an indirect measurement of hydroperoxide, which are intermediate oxidative products of lipids, amino acids and peptides and therefore useful measure of OS. It is a colorimetric test based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxide into derivative radicals. These derivative radicals are then preferentially trapped by a suitably buffered chromogen: 4-Amino-N-ethyl-N-isopropylaniline hydrochloride and develop, in a linear kinetic based reaction at 37°C, a colored fairly long-lived radical cation spectrophotometrically detectable at 505 nm. The intensity of the color correlates directly with the quantity of radical compounds, which is related to the oxidative status of the sample [27].

FORD test provides an estimation of the overall antioxidant capacity of blood plasma. This test is based on the ability of antioxidants present in plasma to reduce a preformed radical cation. A stable colored cation (photometrically detectable at 505 nm) is formed in the presence of an acidic buffer (pH = 5.2) and an oxidant ($FeCl_3$). Antioxidant compounds present in the analyzed sample, reduce the radical cation of the chromogen, quenching the color and producing a discoloration of the solution, which is proportional to their amount in the sample [27].

To examine the extent of muscle damage, venous blood samples were collected from the antecubital vein of athletes in serum separator tube using vacutainer system (Greiner Bio-One, Kremsmünster, Austria). The serum separator tubes were placed on ice and left to stand for 30 minutes to facilitate clotting before being centrifuged at 3500 g for 15 minutes to obtain serum. Creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were determined in a clinical laboratory using current bioassays based on methods by Johnson et al. [28]. LDH activity determination is based on the conversion of pyruvate to L-lactate by monitoring the nicotinamide adenine dinucleotide (NADH) oxidation. AST is assayed in a coupled reaction with malate dehydrogenase in the presence of NADH. In the determination of CK activity, the enzyme reacts with creatine phosphate and adenosine diphosphate to form adenosine triphosphate, which is coupled to the hexokinase/guanosine diphosphate reaction generating NADPH.

Statistical analysis

Statistical analyses were performed with the software IBM SPSS Statistic version 20.0 (IBM Corp., Armonk, NY). All data were assessed for normality (one-sample Kolmogorov–Smirnov test). FORT and FORD were analyzed using two-way analysis of variance (ANOVA) with repeated measures. Significant changes in muscle enzyme activities at rest, as well as values of basic motor skills test obtained at the beginning and at the end of the study were analyzed using paired sample t-tests. Data were expressed as mean ± SD. A p-value < 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics of the basketball players are shown in Table 1. All subjects consumed the appropriate amount of product throughout the study period. None of the subjects reported adverse effects related to the dietary supplement.

Statistically significant difference was not recorded regarding the results of basic motor skills tests after 45-day supplementation period. The obtained results are shown in Table 2.

Basketball specific exercise bout induced significant increase in FORT at the beginning of observational period ($p < 0.05$), (Figure 1). However, these changes were not recorded after 45 days of supplementation. In addition, the FORT significantly decreased after 45-day supplementation period ($p < 0.001$). ANOVA repeated measures re-

vealed significant decrease in FORD over the observational period ($p < 0.01$).

We established the overall OS status of the athletes based on both the FORT and FORD results, according to the manufacturers' direction. Five major profiles: normal status NS, latent OS, compensated OS, at risk of OS and OS in progress – have been depicted [27]. The number of athletes with OS in progress was reduced from 71% (10/14 athletes) to 0% (0/14 athletes) and the number of athletes with NS (2/14 athletes) was increased from 14% to 86% (12/14 athletes) as a result of antioxidant supplementation.

The CK and LDH levels at rest, as indicators of muscle damage, significantly decreased after 45 days of supplementation ($p < 0.05$); while no changes were detected, regarding the AST levels (Table 4).

DISCUSSION

In the present study, female basketball players were supplemented with complex antioxidant supplement GE132® during 45 days. The study was performed just before the start of regular basketball season and after completion of basic conditioning training period. This period was chosen since preseason training is highly demanding for athletes because they are engaged in both frequent and high intensity workouts with little or no time to recover. This training program allows neuromuscular and endocrine systems to adapt after the loads placed to them and potential redox status adaptations occur [7, 9, 29, 30].

The major findings of this study indicate that:

1. single basketball training session can increase OS in trained females;
2. the antioxidant combination treatment with GE132® used in this study can significantly attenuate the rise of blood OS markers and muscle damage after basketball exercise and training;
3. GE132® supplementation does not provide benefit for enhancing motor skills of female basketball players.

Table 1. Characteristics of female basketball players

Variables	Mean ± SD
Age (years)	20.6 ± 2.7
Height (cm)	178.1 ± 7.8
Weight (kg)	72.9 ± 10.9
Body Mass Index (kg/m^2)	22.7 ± 2.2
Percent body fat (%)	20.7 ± 4.5
Years of training (years)	8.2 ± 2.7
Hours per week of training (h)	14.2 ± 4.8

Values are presented as mean ± SD

Table 2. Performance changes before and after supplementation period in female basketball players

Motor skills	Before supplementation	After supplementation	p
Push-ups (N)	31.5 ± 10.2	38.3 ± 10.9	< 0.05
Sit-ups (N)	95.5 ± 50.1	121.3 ± 50.4	n.s.
Squat jump (cm)	22.4 ± 2.9	23.4 ± 2.8	n.s.
Countermovement jump (cm)	27.5 ± 5.6	28.1 ± 4.3	n.s.
Vertical jump (cm)	36.1 ± 7.91	36.77 ± 6.55	n.s.
Right leg jump (cm)	14.7 ± 3.7	15.8 ± 2.8	n.s.
Left leg jump (cm)	15.2 ± 2.9	15.9 ± 2	n.s.
Anaerobic endurance (sec)	75.8 ± 5.7	74.1 ± 4.9	< 0.05
Agility (sec)	11.1 ± 0.5	10.9 ± 0.7	n.s.

Values are presented as mean ± standard deviation; N – number of repetitions; cm – centimeters; sec – seconds

Table 3. Muscle enzyme activities at rest before and after supplementation period in female basketball players

Enzyme levels	Before supplementation	After supplementation	p
AST (IU/L)	21.7 ± 3.6	19.5 ± 2.3	n.s.
CK (IU/L)	143.5 ± 35.6	108.7 ± 32.3	< 0.05
LDH (IU/L)	179.4 ± 14.4	164 ± 25.6	< 0.05

Values are presented as mean ± standard deviation; AST – aminotransferase; CK – creatine kinase; LDH – lactate dehydrogenase

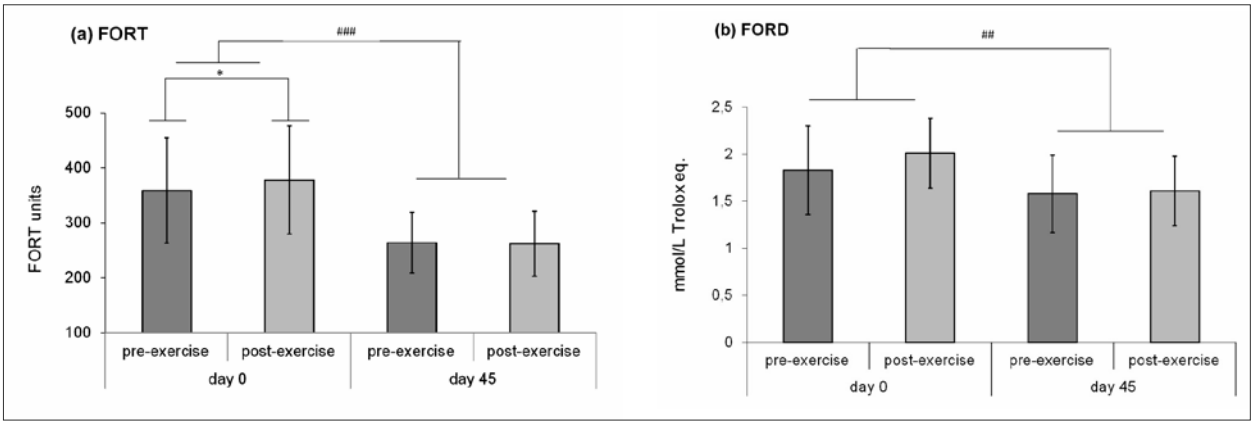

Figure 1. a) The levels of reactive oxygen species measured by the Free Oxygen Radicals test (FORT) before and after supplementation, in pre-exercise and post-exercise conditions; b) the capacity of plasmatic antioxidants Free Oxygen Radical Defense test (FORD), measured by FORD test before and after supplementation, in pre-exercise and post-exercise conditions; values are presented as mean ± standard deviation;

*p < 0.05;
##p < 0.01;
p < 0.001

Based on FORT assay measurement, we found that OS was significantly increased in response to basketball specific exercise bout before supplementation. Basketball is one of the mixed sports that include aerobic phases (intermittent running at different intensity) and anaerobic phases (jumps, sprints). Therefore, the increased free radical generation in basketball can occur via several pathways: mitochondrial respiration, oxidase enzymatic activity (NADPH oxidase, xanthine oxidase), via phagocytic respiratory burst, a loss of calcium homeostasis and/or the destruction of iron containing proteins [2, 31]. The increase in ROS production resulting from any of the above sources, could lead to oxidative changes of different biomolecules, and increased levels of OS. The antioxidant supplementation attenuated this increase after exercise bout at the end of study period, as evidenced by the non-significant changes in FORT levels. Additionally, the overall decreased FORT levels indicate less oxidative damage after 45 days of supplementation. The attenuated OS response is consistent with other studies using antioxidant supplementation [12, 15, 18, 32–36]. On the other hand, similar changes might occur as a result of adaptive response to chronic exercise [7, 9]. However, since present study was conducted after the conditioning preseason training, which allowed redox status adaptations, the reduced OS observed in female basketball players might be the result of antioxidant supplementation alone.

The antioxidant system capacity of plasma, measured by FORD test, depends on individual and synergic effects of different molecules, such as proteins, glutathione, vitamin E, ascorbate, carotenoids, and phenolic compounds. We detected no changes of FORD in response to exercise at the beginning of neither the observational period or at the end. Mobilization of tissue antioxidant stores into the plasma is an accepted phenomenon that would help maintain antioxidant status in plasma at certain level and protect body against ROS [37]. In addition, soluble plasma antioxidants work synergistically to defend against oxidant production, meaning that when one antioxidant nutrient is lacking at a particular period in time, another could substitute or it may be regenerated by another that is in abundance [38]. These rapid, dynamic responses in order to maintain redox homeostasis could be the reason for non-significant changes observed in FORD after the exercise. However, plasma levels of antioxidants, measured by FORD, decreased over the entire observational period in response to supplementation. This may not increase athlete's susceptibility to OS, since supplementation decreased oxidative modifications of various biomolecules, as indicated by FORT test. In addition, OS status of the female basketball players was improved after supplementation, judging by the increased number of athletes with NS and decreased number of athletes with OS in progress. Therefore, this antioxidant supplement may provide protection against the negative health consequences of free radicals produced during training.

Although the mechanisms behind exercise-induced muscle damage are not precisely known, it is believed that along with initial mechanically induced disruption, secondary damage is caused by the free radical production and subsequent OS [39]. Some markers, such as AST, CK, and LDH, have been used as a way to indicate the grade of muscle cell damage, especially after playing a sport, since microfiber breakdown releases cell content [5, 40]. The supplementation with antioxidants significantly reduced plasma muscle enzyme activities (CK and LDH), suggesting the involvement of oxidant mechanisms on tissue injury induced by the exercise. This finding can be explained by protective effect of antioxidants against lipid peroxidation, resulting in less muscle membrane damage. Our results are in accordance with several studies, which reported beneficial effects of antioxidants in terms of minimizing the rise in muscle enzyme activity in response to exercise [16–19, 41, 42].

There has been a general inconsistency of outcomes when investigating the role of antioxidant supplementation in exercise performance, with the majority of the studies reporting no benefits. In accordance, in the present study no statistically significant difference was observed regarding repetitive or explosive strength, endurance, or agility performance after supplementation period in comparison to baseline.

The limitations of the study include the small number of subjects and the short duration of supplementation. However, particular strength of this study is the fact it was conducted during a regular competitive half season, reflecting habitual conditions of nutrition and training program. In addition, this is the first study examining the effects of nutraceutical blend GE132®.

CONCLUSION

Previous studies showed that professional athletes are exposed to increased OS over the periods of intensive training. Exercise-induced OS might be associated with fatigue, muscle damage, and increased recovery time that can all affect exercise performance. For that reason, reliable and quick tests for OS status measurements, such as FORD and FORT assays, might be very useful for training and supplementation planning. Exogenous supplementation with protective nutraceuticals such as those found in GE132®, could reduce acute and chronic OS during high intensity efforts, and provide beneficial effect on muscle function recovery. The results of the present study suggest that GE132® supplementation does not enhance performance of female basketball players, but rather provide protection against detrimental health consequences of ROS produced during training.

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Conflict of interest: None declared.

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Утицај комбиноване суплементације антиоксидансима на оксидативни стрес, оштећење мишића и спортску способност код кошаркашица

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САЖЕТАК

Увод Утврдили смо утицај комбинације антиоксиданата *GE132*® на спортску способност, маркере оксидативног стреса и активности мишићних ензима код професионалних кошаркашица.

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 Поновљена снага, експлозивна снага, анаеробна издржљивост и агилност су мерени пре/после 45-дневног периода суплементације. *FORT* (тест слободних кисеоничних радикала) и *FORD* (тест антиоксидативне заштите) процењени су пре/после специфичног кошаркашког тренинга на почетку/крају периода суплементације. Степен оштећења мишића је процењен мерењем активности аспартат-аминотрансферазе, креатин-киназе и лактат-дехидрогеназе у серуму.

Резултати После суплементације није забележена значајна разлика у резултатима тестова моторичких способности у односу на период пре суплементације. Кошаркашки специфични тренинг је изазвао значајно повећање *FORT*-а ($p < 0,05$) само на почетку периода суплементације. И *FORT* и *FORD* су значајно опали током посматраног периода ($p < 0,001$, $p < 0,01$). Креатин-киназа и лактат-дехидрогеназа су биле значајно ниже на крају периода посматрања ($p < 0,05$) у поређењу са вредностима пре суплементације.

Закључак Суплементација са заштитним нутритивним препаратима, као што су они у *GE132*®, може смањити акутни/хронични оксидативни стрес и оштећење мишића, али није показан утицај на спортске перформансе кошаркашица.

Кључне речи: антиоксиданси, оксидативни стрес; *FORT*; *FORD*; кошарка; оштећење мишића

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Informatics literacy among first-year students of medicine, dentistry and pharmacy at the University of Niš in compliance with European Computer Driving Licence

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SUMMARY

Introduction/Objective Having taken into account the complex role of students and teachers participating in the global education system and the creation of European Higher Education Framework, it was necessary to perform research on Informatics Literacy (IL). Therefore, our aim was to analyze IL of the students including the knowledge of each of the four core modules and two standard European Computer Driving Licence modules, as well as to propose measures to improve students' IL. 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 We conducted a cross-sectional study during 2015/2016 at the Faculty of Medicine, University of Niš, and included 292 first year students. Parts of the questionnaire that related to the self-assessment, as well as the test of knowledge in Informatics, modeled after the ECDL consisted of questions from six thematic sections.

Results The study included 88 male (30.1%) and 204 female (69.9%) students. The computer is mostly used for the Internet (69.5%), then for entertainment (24.3%), and seldom for data processing programs (6.2%). Medical students showed higher level of knowledge of all six modules ($p < 0.001$). Male students also had higher level of IL (OR = 0.38, 95% CI 0.20–0.73). Students who completed high school showed better IL compared to students who completed secondary medical school (OR = 0.34, 95% CI 0.18–0.66). Education of parents and monthly income had no impact on students' IL.

Conclusion IL of students is not satisfactory. It is necessary to modify the Informatics curriculum according to European standards and to introduce the course to all study groups.

Keywords: informatics literacy; students; ECDL

INTRODUCTION

What is informatics literacy?

The need for literacy has changed over time, so that policy and teaching of literacy nowadays is beyond the scope of understanding it in the past. Primary or elementary literacy includes reading and writing skills. Secondary or functional literacy includes understanding of written documents in everyday life (e.g. filling instructions and forms). Tertiary literacy encompasses informatics, computer, Internet, SMS literacy, etc. The development of information technology and its presence in all segments of society has led to increased understanding of broader concept of informatics literacy (IL) which is the basis for the development of modern society [1].

The terms IL and "information literacy" are different. The term "information literacy" refers to the ability to collect, transfer, and process data and use the specific information, while IL (computer literacy) refers to the general ability for work on the computer and use of computer programs [2, 3, 4]. Although nowadays they are considered interlocked, they are not synonymous.

Since the 1990s, there have been frequent discussions about computer literacy as a skill that everyone should adopt in order to be able to work effectively in the modern world. In fact, computers have become an inevitable tool not only in all disciplines and areas, but in everyday life as well. The concept, then, has been expanded to some basic informatics knowledge, and started to include IL. However, there was no defined standard, which could determine the level of knowledge and skills required for IL.

The aim of education is to create conditions for scientific literacy based on developed informatics and information literacy in the process of creating the knowledge society [2, 3]. In the case of higher education, it is necessary to create such conditions that would enable every student to be the part of the system in which new values are deeply rooted in knowledge and there its foundation will be created, grounded in informatics and information literacy.

In the field of higher education it is expected that students and teachers can respond to all elements of IL, which provides education

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with the role of the common good, as proclaimed by the UNESCO Conference on Higher Education in Paris in 2009 [2, 4]. Some of the proclaimed goals include IL set by UNESCO in 2004, the goals of life-long learning and distance learning in higher education from 2005, as well as goals adjusted to Bologna documents on higher education in Europe – Bologna Process, 2010 [2, 4–7].

European Computer Driving Licence Foundation

The European Commission has launched an initiative in 1995 to increase the level of IL in Europe. Thus, in Dublin in 1997 an institution called the European Computer Driving Licence Foundation Ltd. was founded. Rapidly, European Computer Driving Licence (ECDL) was accepted in many European countries and beyond, and was spread as a method for acquiring IL [8].

In 2003, ECDL received official support from the European Commission, and became the official standard in state bodies of the EU member states. ECDL was accepted for a short time outside the EU, so it is now used in 148 countries and in 36 languages. In Serbia, along with efforts to join the EU defined the ECDL and its "Strategy of development of the information society" in 2006 [8].

ECDL curriculum and syllabus include knowledge assessment of several thematic sections or modules, which may be basic, standard, and advanced.

Basic modules represent a set of primary skills that are essential for each individual. By taking examination in all four basic modules, a person obtains ECDL Start certificate. This level includes the following modules consisting of the skills below:

1. Basic computer use – knowledge and skills referring to device use, creation and arrangement of files, network and security aspects;
2. Basics of Internet use – knowledge and skills related to Web search, efficient information finding, online communication and e-mail messages;
3. Text processing – knowledge and skills necessary for efficient use of application for creation, formation and final text processing;
4. Tabular calculations – knowledge and skills necessary for efficient use of applications for creation, formation, changing and use of working sheets, standard formulas, functions and graph creation.

Standard modules represent a set of practical skills used in each of the corresponding areas chosen by candidates themselves according to their needs.at work. There are nine standard modules, and by taking combined examination version with basic modules ECDL Core or ECDL Profile certificate could be obtained. The standard modules include:

(i) Presentations, (ii) Use of data base, (iii) IT safety, (iv) Online collaboration, (v) Image processing, (vi) Web site processing, (vii) Project planning, (viii) 2D Computer Aided Design, and (ix) Use of health information system (Appendix 1).

Advanced modules allow the user to become "a powerful computer user" and use completely four most common applications. ECDL advanced levels are: Advanced text processing, Advanced table calculations, Advanced databases and Advanced presentations.

Appendix 1 – STANDARD MODULES

Standard modules represent a set of practical skills used in each of the corresponding areas chosen by candidates themselves according to their needs at work. There are nine standard modules, and by taking combined examination version with basic modules ECDL. Core or ECDL Profile certificate could be obtained. Standard modules include:

1. Presentations – Formation of professional standard presentation. Creation, formatting, modification and preparation of presentations using different slides for demonstration and distribution.
2. Use of data base – Efficient use of desktop data base, understanding basic concept of data base and demonstrating the ability of using data base application by creating and modifying tables, inquiries forms, reports and preparing them for distribution. Connecting tables, uploading, and manipulating information from data base using inquiries and sorting tools.
3. IT safety – Ensuring personal and organization data safety, secure use of online services including the use of social networks.
4. Online collaboration – Developing concepts and skills relating to adjustment and use of online collaboration tools, social media, webinars, mobile technology, and cloud computing.
5. Image processing – Acquisition of skills and competences relating to different programs for image processing.
6. Web site processing – Understanding basic concepts of web site processing and publishing, design, creation, installation and maintenance of static web page.
7. Project planning – Using project management software. A candidate will be able to prepare a project plan, monitor projects, organize time, costs and tasks.
8. 2D Computer Aided Design – Acquisition of skills for creating and modifying objects or elements in a two-dimension design. Getting acquainted with changing properties, objects, preparation for printing or plotting.
9. Use of health information system – Designed for doctors, nurses, as well as the complete doctor's team caring about patients.

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Appendix 1 – STANDARD MODULES

In accordance with the conclusions of the Commission for education program harmonization with ECDL standards, pupils from elementary or secondary school who obtain very good (4) or excellent grade (5) in Informatics or students at university who get grades 8 (very good), 9 (excellent) or 10 (remarkable) can obtain ECDL certificate. Hereby, the Informatics curriculum has to comply with the ECDL modules in the following way:

If the curriculum includes all four basic modules, then a student can obtain ECDL Start certificate. If, in addition to four basic modules, the curriculum includes standard modules of Presentations and Database use, then a student can obtain ECDL Core certificate [8].

It is exactly this principle that we used in the analysis of IL among first-year students of the Departments of Medicine, Dentistry, and Pharmacy of the Medical Faculty in Niš, taking into account the fact that similar research has not been conducted according to accepted ECDL European standards in our region. However, the main goal of this research was not only to determine the level of IL acquired during secondary education, but also to find optimal solutions that would increase the IL of students during their studies.

Appendix 2 – QUESTIONNAIRE

1) What is your study group?
a) Medicine **b)** Dentistry **c)** Pharmacy

2) Sex
a) Male **b)** Female

3) What secondary school did you complete?
_____

4) Are you satisfied with the Informatics knowledge acquired in secondary school?
a) Yes **b)** No

5) What are your parents' qualifications?

Mother:	Father:
a) Elementary school	**a)** Elementary school
b) Secondary school	**b)** Secondary school
c) College	**c)** College
d) University	**d)** University
e) Master/doctoral degree	**e)** Master/doctoral degree

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6) What is the total monthly income of your household (roughly)?_____

7) How many members are there in your family?_____

8) Do you have a computer at home or temporary residence?
a) Yes (circle the type of computer – more than one answer possible
i) Desktop computer **ii)** Laptop **iii)** Tablet
b) No

9) How many computers are there in your household?_____

10) How old were you when you started using a computer?_____

11) What is the purpose for which you use a computer?
a) Entertainment (playing games, watching movies,...)
b) Internet use
c) Data processing using (Word, Excel, Power Point,...)

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12) How would you assess your knowledge of operation system?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

13) How would you asses your knowledge of Microsoft Office Word?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

14) How would you assess your knowledge of Microsoft Office Excel?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

15) How would you assess your knowledge of Microsoft Office Power Point?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

16) How would you assess your knowledge of Microsoft Office Access?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

17) How would you assess your knowledge of Internet?
a) Not enough **b)** Enough **c)** Good **d)** Very good **e)** Excellent

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18) File with .docx (.doc) extension represents:
a) database **b)** compressed file; **c)** text document; **d)** I do not know.

19) File with .xlsx (.xls) extension represents:
a) table; **b)** presentation; **c)** web document; **d)** I don't know.

20) File with .pptx (.ppt) extension represents a:
a) text document; **b)** video file; **c)** presentation; d) I don't know

21) File with .accdb (.mdb) extension is:
a) video file; **b)** executable file; **c)** database; **d)** I don't know.

22) Which of the following belongs to computer operation system?
a) Avast **b)** Windows 7 **c)** WinZip **d)** I don't know

23) Is it possible to search a document in Word using a key word?
a) Yes **b)** No **c)** I don't know

24) Can functions in Excel be applied to numerical data?
a) Yes **b)** No **c)** I do not know

25) While printing a presentation is it possible to print several slides on one piece of paper?
a) Yes **b)** No **c)** I do not know

26) Is it possible to copy table from Excel to Word document?
a) Yes **b)** No **c)** I do not know

27) Is it possible to insert both text and image on the same slide in Power Point presentation?
a) Yes **b)** No **c)** I do not know

28) In an email address petar10@gmail.com, petar10 represent:
a) password; b) user name; **c)** domain; **d)** I do not know.

29) Which program opens a pdf file?
a) Windows Media Player **b)** Adobe Reader
b) Kaspersky AVP **d)** I do not know

30) Page numbering in Word:
a) cannot be inserted;
b) can be inserted only at the top of page;
c) can be inserted only at the bottom of the page;
d) can be inserted both at the top and bottom of page;
e) I do not know

31) Data sorting in a table created in Access is possible:
a) only using one criterion; **b)** using several criteria;
c) only in increasing order; **d)** I do not know.

32) The function of Outlook Express program is for:
a) creating Web presentations; **b)** Internet access;
c) receiving and sending e mails; **d)** I do not know.

33) Which statement is correct?
a) Operation system provides computer protection from viruses.
b) Operation system represents a sum of all programs on a computer.
c) Operation system is necessary for the use of programs and files.
d) I do not know.

34) One table in Access may have:
a) only one primary key; **b)** multiple primary keys;
c) maximum two primary keys; **d)** I do not know.

35) Modem is:
a) an electronic device enabling Internet access;
b) operation system of a computer by means of which we get connected to Internet;
c) program enabling Internet access;
d) I do not know.

Appendix 2 – QUESTIONNAIRE

METHODS

The research represents a cross-sectional study carried out in academic 2015/2016 at the Medical Faculty of University in Niš. This study was done in accord with standards of the institutional Committee on Ethics. Out of the total number of 336 first year students of integrated studies of Medicine, Dentistry and Pharmacy 292 of them were included in the study. They gave consent to participate in the study and filled in anonymous questionnaire at lectures and practical. Completing of the questionnaire lasted for 15 minutes, including the time for the interviewer's instructions.

Out of the total number of 195 first-year students of the Department of Medicine, 186 were included in the study; 45 students of the Department of Dentistry (out of 71) and 69 students (out of 70) of the Department of Pharmacy were also included in this study. The questionnaire consists of three thematic units (Appendix 2):

- open and closed questions relating to demographic and socioeconomic characteristics;
- closed questions relating to students' self-assessment of their IL;
- closed questions relating to objective assessment of students' IL.

Parts of the questionnaire relating to self-assessment as well as objective assessment of Informatics competence based on the test using ECDL, consisted of questions grouped in six thematic units – modules (Figure 1). The part of the questionnaire relating to the self-assessment of competence in six thematic units (modules) from Informatics contained six questions – one question for each module. These six questions had five possible answers from "not enough" (1) to "excellent" grade (5).

Analysis of self-assessment of "Start" and "Core" information literacy of respondents was done in two ways:

- The first way implied that the respondents possessed a "Start" or "Core" information literacy if they had grade 4 or 5 from each of the program modules.
- The second method involved the so-called. "School assessment type," i.e. that the students "passed" all the modules (if they graded their knowledge for each module ≥ 2) and had 4 as the final grade.

The part of the questionnaire that related to knowledge assessment of all six modules consisted of 18 questions, with three questions for each module. (Questions for assessment of students' IL were made in cooperation with experts from the Faculty of Science of the University in Niš and experienced lecturers of Informatics working at the University in Niš). The evaluation of the number of correct answers per module was carried out in the manner described below.

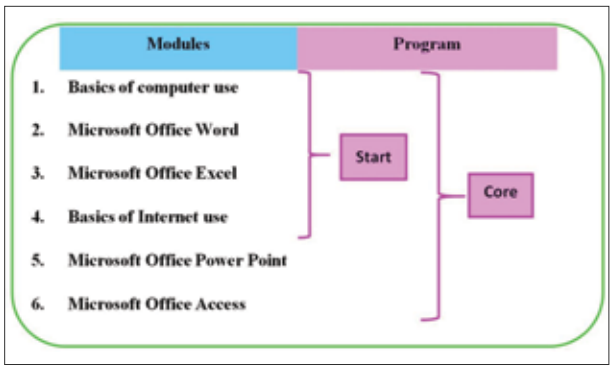

Figure 1. Modules "Start" and "Core"

If a student had no correct answers in a given module, he was estimated not to have knowledge in that module. If he had only one correct answer in a module, he was estimated to have low knowledge level. If he answered two questions correctly, he was estimated to possess average knowledge, and if all three answers were correct, he was estimated to have a very good level of knowledge of a given module.

As with knowledge self-assessment, the analysis of the results of IL through knowledge examination was done in two ways:

- The first way implied that the student was "Start" or "Core" literate if he answered all three questions correctly in all program modules of the program (grades 4 or 5);
- The second method of analysis was based on the school assessment type – if a student "passed" all the necessary modules (i.e. if he has one correct answer), and received the final grade 4, then he is said to be "Start" or "Core" literate (depending on the observed modules).

Data were processed in Microsoft Office Excel 2007 by teams of two people, whereby crosscheck was carried out for each input. The total number of received questionnaires was 301 but nine questionnaires were eliminated due to incomplete responses, so that the final number of processed questionnaires was 292. Of the 35 questions in the questionnaire, some of which contained sub-questions, 23 variables were made.

The respondents were classified in groups in relation to:

- sex
- secondary school completed
- study program

The examined factors were compared between these groups. Statistical analysis was performed in SPSS 17.0 (SPSS Inc., Chicago, IL, USA) in Windows 7 environment. The results were presented in tables. Statistical analysis included the application of descriptive statistics (percentage distribution, mean value, median), parametric tests (Student's t-test), Spearman rank correlation and nonparametric tests (Pearson's χ^2 test, Fischer's exact test) as well as binary logistic regression model. Statistical significance was taken at $p < 0.05$.

RESULTS

Assessment of students' knowledge of modules

Out of the total number of 292 examined students, 88 were male (30.1%) and 204 were female (69.9%). Exactly 184 students (63%) completed secondary medical school (or similar vocational school), and 108 (37%) completed high

Table 1. General overview of respondents

Parameters	Sex		Secondary school		Satisfied with their knowledge from secondary school		Total
Study group	Male	Female	High school	Secondary medical school	Yes	No	
Medicine							
Number	56	126	73	109	90	92	182
%	30.8%	69.2%	40.1%	59.9%	49.5%	50.5%	100%
Dentistry							
Number	16	28	9	35	23	21	44
%	36.4%	63.6%	20.5%	79.5%	52.3%	47.7%	100%
Pharmacy							
Number	16	50	26	40	25	41	66
%	24.2%	75.8%	39.4%	60.6%	37.9%	62.1%	100%
Total							
Number	88	204	108	184	138	154	292
%	30.1%	69.9%	37%	63%	47.3%	52.7%	100%

Table 2. Informatics literacy of students in relation to modules

Self-assessment of knowledge					Assessment of knowledge (test)			
Not enough	Enough	Good	Very good	Excellent	No knowledge	Poor knowledge	Average knowledge	Very good knowledge
Basics of computer use								
46	95	82	50	19	6	44	130	112
15.8%	32.5%	28.1%	17.1%	6.5%	2.1%	15.1%	44.5%	38.4%
Microsoft Office Word								
16	70	86	75	45	17	55	89	131
5.5%	24%	29.5%	25.7%	15.4%	5.8%	18.8%	30.5%	44.9%
Microsoft Office Excel								
56	80	91	49	16	59	97	84	52
19.2%	27.4%	31.2%	16.8%	5.5%	20.2%	33.2%	28.8%	17.8%
Microsoft Office Power Point								
21	59	79	82	51	10	39	98	145
7.2%	20.2%	27.1%	28.1%	17.5%	3.4%	13.4%	33.6%	49.7%
Microsoft Office Access								
122	82	59	21	8	156	89	36	11
41.8%	28.1%	20.2%	7.2%	2.7%	53.4%	30.5%	12.3%	3.8%
Internet								
3	28	72	92	97	14	43	86	149
1%	9.6%	24.7%	31.5%	33.2%	4.8%	14.7%	29.5%	51%

school. As for study courses, there were 182 respondents from the Medicine study program, 44 respondents were from the Dentistry study program, and 66 respondents were from the Pharmacy study program. Exactly 138 (47.3%) respondents were satisfied with the informatics knowledge acquired in secondary school, while 154 (52.7%) students were dissatisfied (Table 1).

Almost all examined students (97.9%) possessed and used a computer (at least one). Only four of them reported that they did not own a computer. The average income per household was 76,129.63 Serbian dinars, with the average number of household members 4.12 (i.e., the average income per household member is 18,487.06 Serbian dinars). The average number of computers per household was 2.25. The average age when respondents started to use computer was 9.59 years.

Respondents most often used their computer for the Internet (69.5%), then for entertainment purposes (playing computer games, watching movies, listening to music, etc.) (24.3%), and for data processing program (6.2%) (MS Word, MS Excel, MS Power Point, etc.).

The results of self-assessed and examined knowledge of all six modules were presented in Table 2. In terms of the order of the number of students who were trained to work in these thematic units, the result was as follows (very good and excellent grades of respondents were taken into account):

1. Internet – 189 respondents (63.7%)
2. Microsoft Office Power Point – 133 respondents (45.6%)
3. Microsoft Office Word – 120 respondents (40.2%)
4. Basics of computer use – 69 respondents (23.6%)
5. Microsoft Office Excel – 65 respondents (22.3%)
6. Microsoft Office Acces – 29 respondents (9.9%)

Correlations between self-assessed and examined knowledge of modules

The correlation between self-assessed and examined knowledge was investigated using Spearman's rank correlation. Preliminary analyses were performed to prove assumptions of normality, linearity, and homogeneity of variance. Strong positive correlations were calculated between these two variables. According to Cohen, correlation strength between these two variables was medium ($0.3 < r < 0.49$) for all these modules except for Microsoft Excel, where correlation was slightly lower (Table 3).

Table 3. Correlation between self-assessed and assessed (by test) knowledge of modules

Self-assessment of knowledge	Assessment of knowledge (test)
	Spearman's ρ
Basics of computer use	0.331**
Word	0.406**
Excel	0.283**
Power Point	0.435**
Acces	0.348**
Internet	0.320**

**p < 0.001

Thus, high levels of subjectively experienced knowledge were accompanied by high levels of objectively examined knowledge.

Overall IL of respondents

Comparison of IL between results obtained by self-assessment and results obtained by knowledge assessment was performed in two ways:

Traditional assessment

According to the model of the ECDL, a student is said to have the basic level of IL ("Start") if he has a very good knowledge of each of the four core modules (basics of computer use, text processing, table calculations, and Internet). If a student had a very good knowledge of each of the six modules, i.e. of all four basic modules and two from the nine standard modules (Presentations and Use of the database), then he was said to have standard level of IL ("Core").

The results relating to the students' knowledge of abovementioned six modules (grades 4 and 5 were taken into account) are as presented in Table 4.

χ^2 test of independence showed a significant difference between the study groups and self-assessed knowledge of "Start" module ($\chi^2 = 6.034$, df = 2, p = 0.049 and Cramer's V = 0.144). Medical students assessed their knowledge as better compared to other two study groups.

χ^2 tests of independence (with continuity correction by Yates) showed significant differences: between sex and self-assessed knowledge of "Start" program ($\chi^2 = 4.805$, df = 1,

Table 4. Start and Core IL according to European Computer Driving Licence standards in relation to study group, sex, and completed secondary school – traditional and school scoring

Parameters	Start				Core			
	Self-assessment		Assessment (test)		Self-assessment		Assessment (test)	
Traditional grading								
Faculty								
Medicine	29	15.93%	17	9.34%	14	7.69%	2	1.1%
Dentistry	2	4.54%	1	2.72%	1	2.72%	0	0%
Pharmacy	5	7.57%	2	3.03%	2	3.03%	0	0%
Sex								
Male	17	19.32%	10	11.36%	9	10.23%	0	0%
Female	19	9.31%	10	4.9%	8	3.92%	2	0.98%
Secondary school								
High school	20	18.52%	10	9.26%	8	7.41%	2	1.85%
Secondary medical school	16	8.7%	10	5.43%	9	4.89%	0	0%
Total	36	12.33%	20	6.85%	17	5.82%	2	0.68%
School grading								
Faculty								
Medicine	74	40.66%	84	46.15%	52	28.57%	40	21.98%
Dentistry	14	31.82%	13	29.55%	11	25%	2	4.55%
Pharmacy	19	28.79%	9	13.64%	11	16.67%	3	4.55%
Sex								
Male	39	44.32%	43	48.86%	22	25%	19	21.59%
Female	68	33.33%	63	30.88%	52	25.49%	26	12.75%
Secondary school								
High school	60	55.56%	57	52.78%	39	36.11%	28	25.93%
Secondary medical school	47	25.54%	49	26.63%	35	19.02%	17	9.24%
Total	107	36.64%	106	36.3%	74	25.34%	45	15.41%

p = 0.028 and φ = -0.14), whereby it was found that male students were more familiar with this program; between high schools and secondary medical schools in relation to self-assessed knowledge of "Start" program (χ^2 = 5.200, df = 1, p = 0.023 and φ = -0.144) in favor of the high school.

School assessment

We used school assessment system for analysis of results following ECDL model. The requirement was that the student obtained minimum grade 2 from each module, (i.e. that he "passed" each module) and at the same time had a very good or excellent final grade from all the modules together (according to self-assessment). In the process of knowledge assessment a student was obliged to have the final grade 4 (whereby he had to have at least one correct answer – i.e. that "he passed" each module).

χ^2 test of independence showed a significant difference between the study group and "Start" program self-assessment (χ^2 = 23.171, df = 2, p = 0.000 and Cramer's V = 0.282), as well as between the study group and the "Core" program of knowledge assessment (χ^2 = 15.983, df = 2, p = 0.000 and Cramer's V = 0.234), in favor of students of medicine in both cases.

χ^2 tests of independence (with Yates' Correction for Continuity) showed significant differences between sex and test-assessed knowledge of "Start" program (χ^2 = 7.836, df = 1; p = 0.005 and φ = -0.172) in favor of the male students.

Testing prognostic values of certain parameters for IL

Binary logistic regression was conducted to assess the impact of various factors on the possibility that students would have good knowledge of Start program. The model contained six independent variables (sex, secondary school, mother's education, father's education, monthly income and the age of starting to use computer) and was statistically significant, χ^2 (5, N = 292) = 33.106, p < 0.001. The model explained variances between 14.3% (r^2 Cox and Snell) and 19.5% (r^2 Nagelkerke) in proven excellent knowledge of Start program and correctly classified 70.2% of cases.

As shown in Table 5, only two independent variables made a statistically significant contribution to a unique model (sex and secondary school). The strongest predictor of the response that a student had excellent knowledge was completed secondary school with OR = 0.34 (95% CI 0.18–0.66). This implied that students who completed high school had three times better knowledge than students from secondary medical or other vocational schools (with all other equal factors in the model). Odds ratio for sex was 0.38 (95% CI 0.20–0.73). Parents' education and income showed no significant association with IL.

DISCUSSION

The research results showed that not all participants had their personal computer, the reason of which lies in a poor economic situation in the region. This represents a sort of disadvantage because in the contemporary informatics era the computer is indispensable tool in all spheres of life and all professions. Therefore, it is desirable that each individual has a personal computer, which would significantly increase computer literacy, improve learning process and general knowledge.

Out of the total number of respondents, 69.5% of them use the computer for Internet access, 24.3% of them for entertainment purposes (playing computer games, watching movies, listening to music, etc.), and only 6.2% of them for data processing programs (MS Word, MS Excel, MS Power Point, etc.). Thus, it could be concluded that only a small number of students use programs for word processing, tabular calculations and making presentations, where no significant differences were found in relation to secondary school completed.

Generally, the students' IL is not satisfactory. Based on the self-assessment, the number of students having an elementary computer literacy according to ECDL standard is 12.33% of the total number. When testing their knowledge even lower results (only 6.85%) were obtained of basic IL. Only the knowledge of Microsoft Office Word was satisfactory.

It may be possible to explain that those students use it most when it comes to faculty work (writing seminar papers, etc.), while other software tools are rarely in use.

There is a significant difference in the knowledge of all six test modules among students from different study groups indicating that medical students are more competent compared to dental and pharmacy students. Male students have a higher level of literacy in relation to female students. Students who completed high school show better

Table 5. Probability prediction of certain parameters for European Computer Driving Licence Start program – test-assessment by school grading system

Parameters	B	Stand. error	Wald	df	p	OR	95% CI for EXP(B)	
							Lower	Upper
Sex	-0.964	0.335	8.294	1	0.004	0.381	0.198	0.735
Secondary school	-1.078	0.336	10.278	1	0.001	0.340	0.176	0.658
Educational background of a mother	0.135	0.172	0.621	1	0.431	1.145	0.818	1.602
Educational background of a father	0.151	0.180	0.708	1	0.400	1.163	0.818	1.654
Monthly income	0.000	0.000	0.081	1	0.776	1.000	1.000	1.000
The start year of computer use	-0.012	0.065	0.034	1	0.854	0.988	0.870	1.123
Constant	-0.073	0.884	0.007	1	0.934	0.930		

Hosmer and Lemeshow test: χ^2 = 4.078, df = 8, p = 0.850 (> 0.05)
B – coefficient for the constant ("intercept") in the null model; OR – odds ratio; Wald – Wald χ^2 test; Exp(B) – exponentiation of the B coefficient (odds ratio); df – the degrees of freedom for each variable

IL compared to those who completed secondary medical (or other vocational school). Students who are satisfied with the knowledge of informatics acquired in secondary school have a significantly higher level of IL compared to those who are not satisfied with the knowledge of informatics acquired in secondary school. Possible explanation should be the difference in the quality of teaching in these schools. Educational background of parents and monthly income had no impact on IL. This may be explained by the fact that the development of computer literacy takes place predominantly under the influence of the school system, while the influence of the family environment is low.

Unlike IL, students' health literacy relates to their personal background and educational path. There was a positive correlation between health literacy and the educational level of the students' parents. A statistically significant relationship was found between health literacy and type of school, family income, and parents' education level [9, 10].

IL including different program areas was also analyzed by several authors from Serbia. Miletić and Grga [11] examined IL among students of the School of Dental Medicine in Belgrade, but used a questionnaire, which was not made according to the modules of ECDL. Zejnilagić-Hajrić et al. [2] explored IL and the use of computers in the classroom on a sample of students of Chemistry and Physics at the University of Sarajevo, but the methodology of this study was not based on the principles of ECDL. Obradović [12] analyzed IL among doctors and nurses at the Clinical Center of Montenegro. The respondents in his study had ECDL certificate.

A few authors from different parts of the world who dealt with IL, computer skills and different aspects of computer use among various groups of subjects had results similar to ours [13–17]. In 1993, Debehnke and Valley [18] examined the IL of Emergency medicine students by means of a questionnaire that consisted of questions related to self-assessment of knowledge of particular application programs and computer skills. Bediang et al. [19] examined the IL and e-learning of students and lecturers at the departments of Medicine, Dentistry and Pharmacy at an African university. Ranasinghe et al. [20] examined the IL of first year medical students at the University of Sri Lanka, and they found that the most important predictor for computer literacy is previous IT training.

There are findings also similar to ours in some previous studies [21–24]. There has also been a large number of studies on IL of nurses and technicians [25–28]. Nevertheless, biomedical students are not expert computer users. Despite an upbringing in a digitized world, many students still lack some basic computing skills [29, 30]. Recent surveys in different countries have also declared that biomedical sciences students' IL was unsatisfactory [31, 32, 33]. IL skills have become a necessity and an integral part of preparing tomorrow's doctors to be sufficiently competent to use informatics resources effectively and efficiently for the best practice of biomedical sciences [34, 35].

The goal of all education systems is to improve the learning process, and educators need to know the educational strategies, methods and procedures, as well as the ways in which students learn. New technological developments are reflected directly in the educational system, and the way of their application and implementation depends on informatics and communication technologies [23, 36]. As the communication technologies are inevitable in all spheres of life and work, all students should be well prepared to apply informatics technologies in the future workplace.

The current state of equipment and practical instruction of Informatics module at the Faculty of Medicine in Niš

In 2010, the computer network of the Faculty of Medicine had about 350 computers located at institutes, amphitheaters, and classrooms. In this way, teachers and teaching associates could perform teaching in a more modern way, and have an access to numerous specialized databases. At that point, the Computer Centre was an integral part of a worldwide network and with its communication, equipment completely satisfied European standards.

Considering that average life span of computers is five years, computers at the Faculty of Medicine no longer represent adequate equipment for practical training of students. Due to low RAM memory, they cannot support new versions of operating systems and more demanding programs cannot be carried out at necessary speed. All the above-mentioned facts indicate that it is not possible to carry out instruction at a satisfactory level.

CONCLUSION

Generally, students' elementary IL is not satisfactory. Medical students are more competent, compared to dental and pharmacy students, and male students have a higher level of literacy than female ones. Students who completed high school show better IL compared to those who completed specialized schools. Educational background of parents and monthly income had no impact on IL.

Proposed measures

It is necessary to modify the curriculum and syllabus of the Informatics course and make it compatible with practical work in computer classroom, as well as to include new modules in the curriculum along with the modernization of computer equipment at the Faculty (as well as in high schools). In addition, the curriculum of the Informatics course should be adjusted to European standards – ECDL program with the introduction of the elective or compulsory Informatics course for all study groups.

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Информатичка писменост студената прве године студија медицине, стоматологије и фармације на Универзитету у Нишу у складу са Европском рачунарском дипломом (*European Computer Driving Licence*)

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САЖЕТАК

Увод/Циљ Циљ рада је анализа информатичке писмености испитиваних студената преко познавања сваког од шест основних модула и два стандардна модула Европске рачунарске дипломе (*ECDL*), као и утврђивање предлога мера за унапређење информатичке писмености студената.

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

Методе Студија пресека спроведена је у току школске 2015/2016. године на Медицинском факултету Универзитета у Нишу и њоме су обухваћена 292 студента прве године интегрисаних академских студија медицине, стоматологије и фармације. Делови упитника који су се односили на самопроцену, као и на проверу знања из информатике, према узору на *ECDL,* састојали су се од питања из шест тематских целина – модула.

Резултати Испитивано је 88 младића (30,1%) и 204 девојке (69,9%). Рачунар користе најчешће приликом употребе интернета (69,5%), затим у сврху забаве (24,3%), а најређе за употребу неких од програма за обраду података (6,2%). Међу студентима различитих студијских група постоји значајна разлика у познавању рада свих шест испитиваних модула и то у корист студената медицине ($p < 0,001$). Студенти мушког пола имају већи степен информатичке писмености у односу на студенте женског пола (*OR* = 0,38, 95% *CI* 0,20–0,73). Студенти који су завршили гимназију показују бољу информатичку писменост у односу на студенте који су завршили средњу медицинску школу (*OR* = 0,34; 95% *CI* 0,18–0,66). Стручна спрема родитеља, као и месечна примања нису показали да имају утицаја на информатичку писменост студената.

Закључак Основна информатичка писменост студената није на задовољавајућем нивоу. Неопходно је модификовати наставни план из Информатике према европским стандардима, уз увођење предмета на свим студијским групама.

Кључне речи: информатичка писменост; студенти; *ECDL*

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Clinical applications of BioAggregate in pediatric dentistry – case reports

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SUMMARY

Introduction Calcium-silicate-based, nanoparticle-sized BioAggregate is produced as an alternative version of mineral trioxide aggregate (MTA). It contains additives such as calcium phosphate and silicon dioxide but does not contain aluminium oxide and bismuth oxide. Studies have shown that BioAggregate's calcium-ion release is better than these qualities in MTA concerning fracture and acid resistance, biocompatibility and sealing ability.

Case outline In this paper, we examine eight case reports. These reports describe the long-term results of using BioAggregate in areas such as pulpotomy and root canal treatment in primary and permanent teeth, partial pulpotomy, artificial apical barrier construction of permanent teeth, root resorption repair, and treatment of *dens in dente*.

Conclusion As evidenced by the case reports examined here, BioAggregate can be used as alternative material to MTA in many dental treatments. These reports also show that the biocompatibility, antibacterial properties, hardening when moisture is present, ideal expansion percentage, impermeability, and dentine adhesion features of BioAggregate provide advantages in clinical use.

Keywords: pulpotomy; root canal treatment; nanoparticle-sized BioAggregate; calcium silicate

INTRODUCTION

Ceramics are some of the oldest synthetic materials based on natural resources. Bioceramics are specially designed ceramics used in medicine, including dentistry, to repair, restructure or replace damaged or injured body organs [1, 2]. Bioceramics consist of polycrystalline ceramics (alumina and hydroxyapatite), bioactive glass, bioactive glass ceramics, or bioactive composites (polyethylene-hydroxyapatite) [3].

Bioceramics have very attractive properties for medicine and dentistry. For dental practices, they have two important advantages. First, bioceramics are biocompatible, nontoxic, shrinkproof, and chemically stable in the biological environment. For example, bioceramics do not produce inflammatory tissue responses when extruded to periodontal tissues during the root repair process. This is due to the hydroxyapatite formed during the material's dentin bonding [4]. Second, they exhibit a strong antibacterial quality given their high pH (12.9) on curing.

Mineral trioxide aggregate (MTA) was the first bioceramic material successfully used in in dental endodontic practices [5]. Given its biocompatibility, superior physical and chemical properties, it has become the preferred material of choice in areas such as perforation repair, retrograde filling, vital pulp treatment, and root-canal treatment of teeth with an open apex [6]. On the other hand, MTA has limitations. These include its long setting time, manipulation difficulty, high cost and tooth discoloration. These deficiencies have prompted the development of alternative materials for the uses described above.

Calcium-silicate-based, nanoparticle-sized BioAggregate (Innovative BioCeramix, Inc., Burnaby, Canada) is produced as an alternative version of MTA in Canada [7, 8]. It is like MTA in many respects. It contains additives such as calcium phosphate and silicon dioxide but does not contain aluminium oxide or bismuth oxide. In addition, studies have shown that its calcium-ion release, acid and fracture resistance, biocompatibility and sealing ability are better than for MTA [8–12].

Despite the positive *in vitro* results, the studies on the clinical use of BioAggregate are limited. Therefore, the aim of this paper is to show the clinical use of BioAggregate in different instances in pediatric dentistry.

CASE REPORTS

The treatments described in the cases presented here were performed at the Faculty of Dentistry, Department of Pediatric Dentistry of the Eskisehir Osmangazi University in Turkey after written informed consent was obtained from the patients' parents. BioAggregate was used as a biomaterial in all of these cases.

Case 1. Pulpotomy in primary teeth

A four-year-old girl who applied for a regular check-up at our clinic was found to have deep dentin caries in the mandibular left primary

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second molar (Tooth 85) (Figure 1a). According to her parents, she had experienced no spontaneous tooth pain. The intraoral examination found no percussion/palpation sensitivity or mobility. The oral mucosa was normal. In addition, there was no pathology found in the tooth's periapical tissues. Nevertheless, the development of the mandibular left permanent second premolar (Tooth 45) follicle was found delayed. Due to the pulp exposure without caries during the cavity preparation and the possibility of not forming permanent premolar (Tooth 45), a pulpotomy was performed using BioAggregate (Figure 1b). During the 24-month follow-up, Tooth 85 was found to be clinically asymptomatic, and the radiographic examinations showed no periodontal/periapical pathology (Figure 1c).

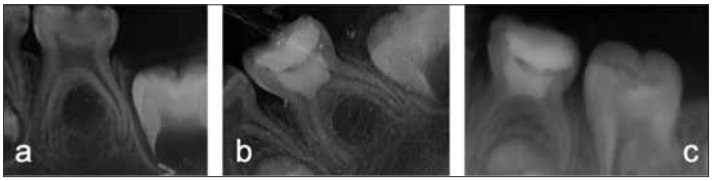

Figure 1. Radiographic appearance of case 1; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

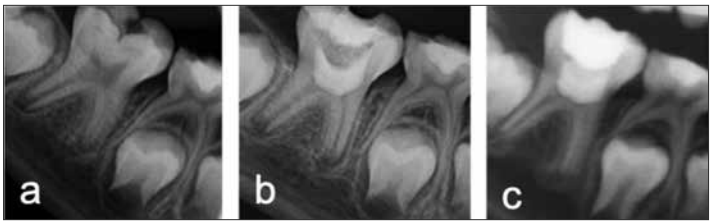

Figure 2. Radiographic appearance of case 2; a – preoperative; b – postoperative; c – postoperative 12-month follow-up

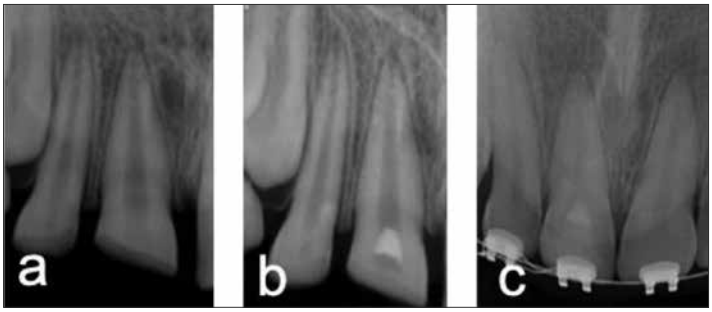

Figure 3. Radiographic appearance of case 3; a – preoperative; b – postoperative; c – postoperative six years follow-up

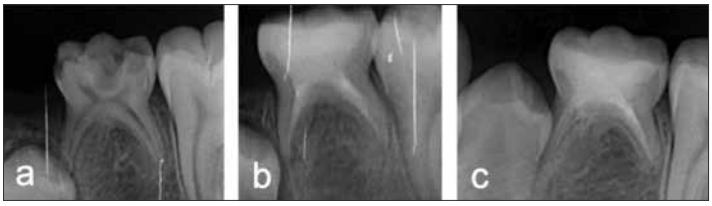

Figure 4. Radiographic appearance of case 4; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

Case 2. Pulpotomy in permanent teeth

An eight-year-old male patient was admitted to our clinic and it was determined to have deep dentin caries in the mandibular right permanent first molar (Tooth 46) (Figure 2a). There was no spontaneous pain, percussion/palpation sensitivity, or pathologic mobility. The oral mucosa was also normal. The radiographic examination revealed that the mandibular right first molar's root development was not complete and that there was no pathology in the periapical region. A pulpotomy procedure was performed with BioAggregateon the tooth, which had responded positively to the vitality test (Figure 2b). In the follow-up radiographic examination at 12 months, Tooth 46 was observed to be vital and showing evidence of continuing root development with no periodontal/periapical pathology (Figure 2c).

Case 3. Partial pulpotomy

A 10-year-old girl was referred to our clinic with a complaint of a crown fracture that occurred when she fell at school. During the clinical examination, an enamel-dentin fracture with pulp exposure was observed in the maxillary right permanent central incisor (Tooth 11). Tooth 11 responded positively to the electric pulp test and exhibited sensitivity to cold and heat. Pathological mobility and percussion/palpation sensitivity were not observed. The radiographic examination found that Tooth 11 showed nearly complete apex formation and that there was no alveolar fracture or any other injury in the apical region (Figure 3a). A partial pulpotomy was performed using BioAggregate (Figure 3b). Throughout the six-year follow-up, Tooth 11 exhibited no clinical pathology or coronal discoloration, and the pulp was observed to be vital. The radiographic examinations showed a closed apex and a dent in bridge at the pulpotomy site (Figure 3c).

Case 4. Root canal treatment in primary tooth

A nine-year-old male patient was admitted to our clinic with severe spontaneous pain in his mandibular left primary second molar (Tooth 75). The intraoral examination found increased percussion sensitivity and mobility. The oral mucosa was normal. The radiographic examination found that the periodontal space of the corresponding tooth was enlarged and that the permanent tooth was congenitally deficient (Figure 4a). A root canal treatment was performed on the devitalized tooth using BioAggregate (Figure 4b). During the 24-month follow-up, Tooth 75 was found to be clinically asymptomatic, and the radiographic examinations showed no periodontal/periapical pathology (Figure 4c).

Case 5. Root canal treatment in permanent tooth

A 10-year-old female was referred to our clinic with complaints of tooth fracture and pain after falling from a bicycle.

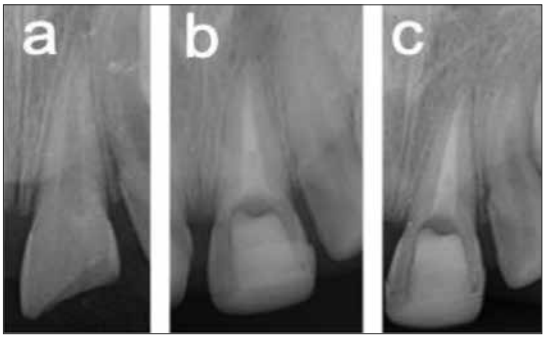

Figure 5. Radiographic appearance of case 5; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

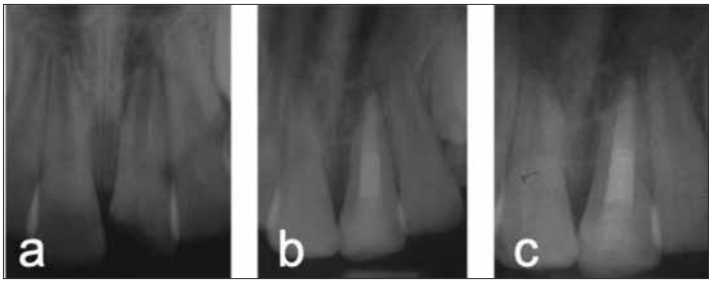

Figure 6. Radiographic appearance of case 6; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

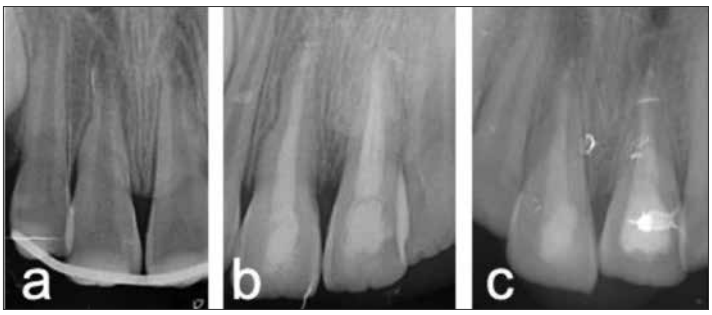

Figure 7. Radiographic appearance of case 7; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

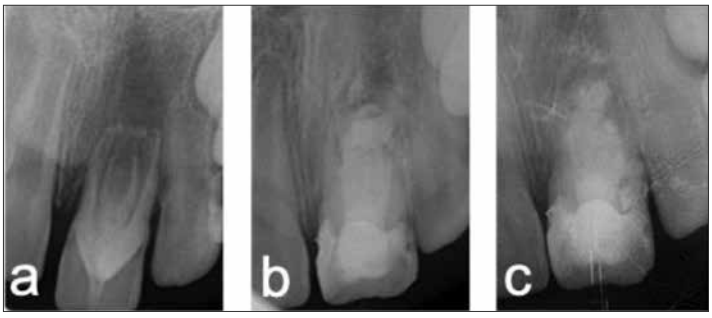

Figure 8. Radiographic appearance of case 8; a – preoperative; b – postoperative; c – postoperative 24-month follow-up

The intraoral examination found a complicated crown fracture of the maxillary right permanent central incisor (Tooth 11) that exhibited spontaneous pain, percussion/palpation sensitivity, and a negative vital response. The radiographic evaluation revealed an enlarged periodontal space and completed root development with a closed apex (Figure 5a). A root canal treatment was performed on the devitalized Tooth 11 using BioAggregate (Figure 5b). Throughout the 24-month follow-up, Tooth 11 was found to be clinically asymptomatic and showed normal color. The radiographic examinations showed no periodontal/periapical pathology (Figure 5c).

Case 6. Artificial apical barrier construction in permanent teeth

A 10-year-old male was admitted to our clinic with a complaint of a crown fracture caused by a fall. In the intake interview, it was learned that the patient was experiencing severe spontaneous pain in the maxillary left permanent central incisor (Tooth 21) that had started two days prior to the visit. The intraoral examination revealed a complicated crown fracture and increased percussion sensitivity in Tooth 21. The radiographic examination showed an enlarged periodontal space, a periapical lesion, and an incomplete root with immature apex (Figure 6a). A root canal treatment was performed to create an artificial apical barrier in Tooth 21using BioAggregate (Figure 6b). Throughout the 24-month follow-up, the tooth showed no clinical pathology or coronal discoloration. The radiographic examinations showed no periodontal/periapical pathology (Figure 6c).

Case 7. Root resorption repair in permanent teeth

An 11-year-old female was admitted to our clinic complaining of dental pain. In the patient's history, we learned that she was in a traffic accident two months prior to visit, and that a splint was performed at another health center because of the teeth mobility. The intraoral clinical examination found that the splint was still in her mouth and that a root canal treatment had been started in the maxillary permanent incisors (Teeth 11 and 21). Nevertheless, the exam found that the patient was still experiencing spontaneous dental pain and percussion sensitivity. The radiographic evaluation showed an enlarged periodontal space, external root resorption areas, and an immature apex (Figure 7a). A root canal treatment was performed using BioAggregate (Figure 7b). During the 24-month follow-up, Teeth 11 and 21 were found to be clinically asymptomatic and showed normal color. The radiographic evaluations revealed that the external root resorptions had been controlled and that no periodontal/periapical pathology had occurred (Figure 7c).

Case 8. Treatment of *dens in dente*

A seven-year-old boy was referred to our clinic with a complaint of dental pain. The intake interview revealed learned that the severe dental pain had started two weeks

before the visit. The intraoral examination detected percussion/palpation sensitivity, pathologic mobility, swelling in the vestibular mucosa and a negative response to the electric pulp test in the left upper central incisor (Tooth 21). The radiographic evaluation revealed an open apex, extensive radiolucency of the periapical tissue and type 3 *dens in dente* (Figure 8a). A root canal treatment was performed using BioAggregate (Figure 8b). Throughout the 24-month follow-up, Tooth 21 was found to be clinically asymptomatic and showed normal color. The radiographic examinations showed no periodontal/periapical pathology (Figure 8c).

DISCUSSION

The use of calcium-silicate-based bioceramics has been steadily increasing due to the negative clinical characteristics of the MTA that is used widely in biomimetic-based treatments. Bioceramics have found place as alternative materials in clinical use not only because of their ductal patency but also for they showed positive results in vital treatments such as pulpotomy and direct pulp capping, apical plugging and retrograde filling [4].

Among these materials, the calcium-silicate-based BioAggregate has specific advantages. First, it is preferred because of its strong physical properties and because it is highly biocompatible, nontoxic, shrink-proof and chemically stable within the biological environment. Second, when extruded to periodontal tissue, it does not cause inflammation, which is very important in endodontic applications [13]. It was also observed that bioceramics can be used as an alternative paste for root canal treatments of open apexes or perforated teeth due to positive properties such as its biocompatibility, and its ability to increase cementoblastic and osteoblastic activity [14, 15, 16].

A further advantage of BioAggregate is that it creates a chemical bond between dentin and filler material by forming hydroxyapatite during the setting process. The resulting hydroxyapatite-like structure acts as a graft material that takes up bone. A significant component of BioAggregate's improving this adherence to the canal wall is its hydrophilic nature and low surface tension. These qualities ensure a high closure for the cover [17]. In addition, it has been shown that the presence or absence of a smear layer does not affect the adherence to the canal wall [18].

A significant portion of BioAggregate's composition is tantalum oxide, which is used instead of the bismuth oxide used in MTA for radiopacity [19]. BioAggregate has a 3.8 mm aluminium equivalent radiopacity, which is higher than MTA's radiopacity [17]. For this reason, it is considered an alternative material because of its advantages in the evaluation of restoration quality [20].

Bioceramic pastes such as BioAggregate exhibit biological activity that includes an alkaline pH (pH > 12), high calcium-ion release, and hydroxyapatite formation. In addition, BioAggregate's tantalum oxide content contributes to antimicrobial activity. Such activity helps prevent failures in pulpal and endodontic treatments in cases involving coronal and apical leakage of microorganisms [16].

In this manuscript, we have shown eight different uses of BioAggregate that achieved successful long-term outcomes. No apical pathology occurred because of these treatments. In addition, as reported in a study by Tuloglu et al. [21], no coronal discoloration was observed in these instances because BioAggregate, unlike MTA, does not contain metal oxides.

That being said, we note that traditional root canal disassembly techniques do not completely remove bioceramics from root canals and that this requires further dismantling of the canal filler. The need for this procedure and the additional time it requires are considered the greatest disadvantage of these materials [22]. We also note that there are limited clinical long-term studies on the performance of this newly developed repair material as an alternative to MTA. Such studies should be done to achieve a greater understanding of BioAggregate's properties and performance.

In conclusion, BioAggregate could be used as an alternative material in many clinical dental treatments. Its biocompatibility, antibacterial properties, hardening in the presence of moisture, ideal expansion percentage, impermeability and dentine adhesion features provide the described advantages in clinical uses.

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Conflict of interest: None declared.

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Примена клиничких биоагрегата у стоматологији – приказ болесникâ

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САЖЕТАК

Увод Биоагрегат (*BioAggregate*) на бази калцијум-силиката, величине наночестица, произведен је као алтернативна варијанта минералног триоксидног агрегата. Садржи адитиве као што су калцијум-фосфат и силицијум-диоксид, али не садржи алуминијум-оксид и бизмут-оксид. Истраживања су показала да Биоагрегат ослобађа јоне калцијума који су отпорни на ломљење и киселину, биокомпатибилни су и непропустљиви, за разлику од својстава минералног триоксидног агрегата.

Приказ болесникâ У овом чланку испитујемо осам приказа болесника. Ови случајеви објашњавају дугорочне резултате употребе Биоагрегата у подручјима као што су пулпотомија и лечење коренских канала примарних и трајних зуба, делимична пулпотомија, уметна апикална баријерска структура трајних зуба, поправљање ресорпције корена и лечење *dens in dente*.

Закључак Као што се види из овде прегледаних случајева, Биоагрегат се може користити као алтернативни материјал минералном триоксидном агрегату у многим стоматолошким третманима. Ови извештаји такође показују да биокомпатибилност, антибактеријска својства, стврдњавање у влажном окружењу, идеалан постотак својстава експанзије, непропустивости и протетског адхезијског својства пружају предности у клиничкој примени.

Кључне речи: пулпотомија; лечење коренских канала; Биоагрегат величине наночестица; калцијум-силикат

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Spontaneous intramural hematoma of the duodenum secondary to anticoagulant drug intoxication

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SUMMARY
Introduction Duodenal hematomas are commonly traumatic, caused by blunt abdominal trauma. Nontraumatic spontaneous intramural hematomas of the duodenum are rare, and in most cases induced by anticoagulant therapy. The diagnosis is based on clinical and biochemical parameters, endoscopy, and radiological examinations. The objective of this report was to present a clinical and radiological presentation of an intramural duodenal hematoma caused by anticoagulant therapy.
Case outline A 43-year-old female was presented with epigastric pain, nausea, hematemesis, and melena. She had a positive medical history of deep venous thrombosis of the pelvis, which was diagnosed the previous month, for which she received oral anticoagulant therapy (coumarin). Physical examination revealed diffuse abdominal tenderness, while laboratory analyses showed markedly elevated international normalized ratio (INR) of prothrombin time (INR > 7), which indicated the anticoagulant intoxication. Computed tomography (CT) showed luminal narrowing with uniform circumferential wall thickening of the descendent and horizontal part of duodenum, which was hyper dense in the native series and slight and uniform ring-formed enhancement in post-contrast phases, a typical CT presentation of intramural duodenal hematoma. Periduodenal and right sided pararenal hematomas were also visualized. After stopping the anticoagulant therapy and performing conservative treatment (vitamin K) with good therapeutic effect being monitored by physical examination, laboratory analyses and transabdominal ultrasonography, spontaneous resolution of the duodenal hematoma was revealed by follow-up CT examination two weeks after the onset.
Conclusion Ultrasonography and CT are useful diagnostic tools in recognition of the intramural duodenal hematoma and other locations of hemorrhage and in monitoring therapeutic effects.
Keywords: duodenal hematoma; anticoagulant intoxication; ultrasonography; computed tomography

INTRODUCTION

Intramural hematoma of the duodenum commonly is traumatic, caused by a blunt abdominal trauma [1]. Nontraumatic spontaneous intramural hematomas are rare, and most cases have been induced by anticoagulant therapy [2, 3]. Such case was reported first time in 1838 [4]. Duodenum is the rarest site of the spontaneous intramural hematoma of the small bowel (about 10%) in comparison to the jejunum and ileum [5, 6]. This condition can be life threatening if bleeding is massive so a rapid and accurate diagnosis is required. The diagnosis is based on clinical and biochemical parameters, endoscopy and radiological examinations such are X-ray contrast upper gastrointestinal examination, endoscopic ultrasound (EUS), transabdominal ultrasonography (US), computed tomography (CT) or magnetic resonance imaging (MRI). Early identification by using the CT is very important in order to promptly stop the oral anticoagulant therapy and introduce the antagonists of warfarin, which would allow avoiding the surgical treatment [7]. The objective of this report was to present clinical and radiological presentation of intramural duodenal hematoma caused by anticoagulant therapy.

CASE REPORT

A 43-year-old female patient was presented with the epigastric pain, nausea, haematemesis and melena. She had a positive medical history of deep venous thrombosis of the pelvis one month ago, why she has received cumarin anticoagulant therapy (acenocoumarol). Physical examination revealed diffuse abdominal tenderness without defense and subcutaneous hematoma of the left lower leg with the presence of melena on digital rectal examination. Laboratory analyses showed leukocytosis (24×10^9/L), anemia (hemoglobin 113 g/L, MCV 82), elevated C-reactive protein (243 mg/L) and markedly elevated international normalized ratio (INR) of prothrombin time (INR > 7), which indicated the anticoagulant intoxication (reference range of INR is < 1.3). Upper flexible endoscopy demonstrated narrowed lumen of the descendent duodenum with the intact mucosa.

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Transabdominal US showed distended stomach and circumferential, uniformly hypoechoic duodenal wall thickening with the periduodenal fat stranding and small amount of fluid in the hepatorenal recess (Figure 1). Abdominal CT examination was performed using the following protocol: after the ingestion of 1.5 L of water, the three-phase CT scanning was performed (native phase and late arterial and portal venous post-contrast phase). CT showed luminal narrowing with uniform circumferential wall thickening of the descendent and horizontal part of the duodenum, which was hyper dense in the native series and slight and uniform ring-formed enhancement in the post-contrast phases, a typical CT presentation of intramural duodenal hematoma. Periduodenal and right-sided pararenal hematomas were also visualized. CT revealed luminal narrowing with the uniform circumferential wall thickening of the descendent and horizontal part of the duodenum (Figure 2). Thickened duodenal wall was hyperdense in the native (Figure 3) and both post-contrast phases, with mild and uniform post-contrast attenuation of the whole wall and marked hyperattenuation of the mucosal layer (Figure 4). Periduodenal strips were propagated towards the right anterior pararenal fascia, hepatorenal recess, mesentery, and recto-uterine recess (Figure 4). According to the CT presentation, the diagnosis of the intramural duodenal hematoma together with the periduodenal hematoma and right perirenal hematoma was established (Figures 2, 3, and 4).

After stopping the anticoagulant therapy and performing conservative treatment (vitamin K was given to reverse the anticoagulant effect of coumarin) with the good therapeutic effect being monitored by laboratory analyses and transabdominal US, spontaneous resolution of the duodenal hematoma was revealed by follow-up CT examination two weeks after the onset.

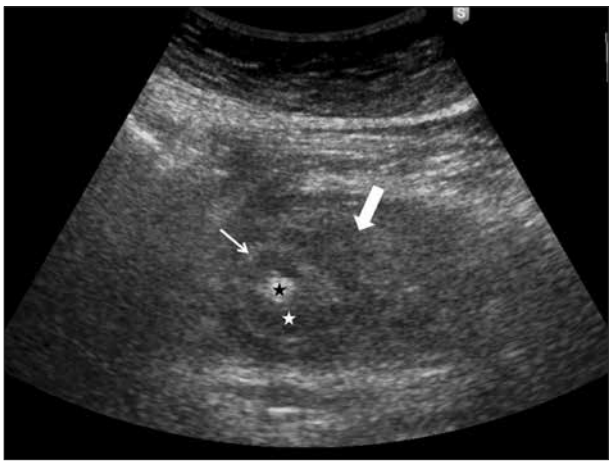

Figure 1. Transabdominal ultrasonography – axial ultrasound scan in the right hypochondrium: thickened and homogenous hypoechogenic D2 duodenal wall (white star, thin arrow), with the hypoechogenic periduodenal mass (thick arrow), represents intramural and periduodenal hematoma (black star indicates narrowed duodenal lumen)

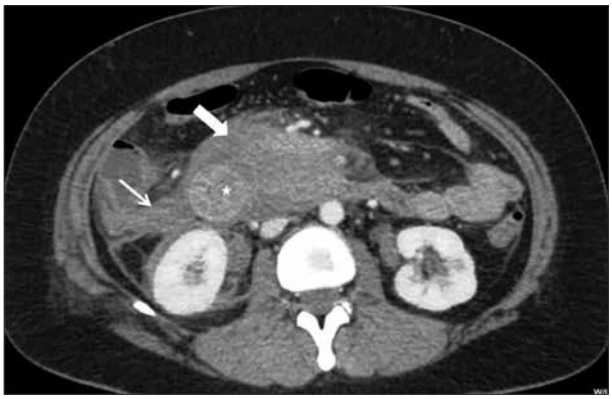

Figure 2. Contrast-enhanced abdominal CT – axial scan: thickened and predominantly hyperdense D2 duodenal wall (white star), with luminal narrowing and hyperdense periduodenal mass (thick arrow), represents intramural and periduodenal hematoma; also notice right perirenal hematoma (thin arrow)

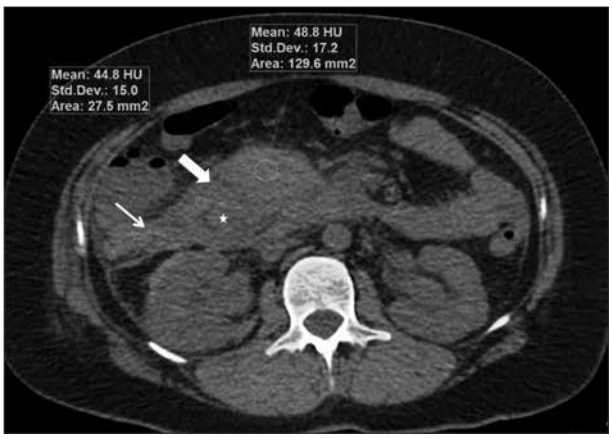

Figure 3. Non-contrast abdominal CT – axial scan: thickened and hyperdense D2 duodenal wall (average attenuation 44.8 HU) (white star: lumen of the duodenum), with hyperdense periduodenal mass (thick arrow; average attenuation 48.8 HU), represents intramural and periduodenal hematoma; also notice right perirenal hematoma (thin arrow)

DISCUSSION

Spontaneous intramural duodenal hematoma is usually associated with coagulation factors' abnormalities resulting from anticoagulation drugs [8]. The vitamin K antagonist warfarin remains a primary agent for oral anticoagulation in the treatment of thromboembolic disorders [9]. Due to its extensive interpatient variability and narrow therapeutic range, warfarin requires frequent laboratory monitoring by INR testing and close patient follow-up in order to prevent adverse effects of therapy such is spontaneous bleeding [10]. Hemorrhagic complications of the anticoagulant therapy could be manifested by hematuria, gastrointestinal hemorrhage, cerebral hemorrhage, soft tissue hematoma, epistaxis, and retroperitoneal hematomas [11]. An incidence of a spontaneous intramural hematoma of the duodenum of one per 2,500 patients who receive the warfarin-based anticoagulant therapy was reported [12]. To the best of our knowledge, this is the first case of a spontaneous intramural duodenal hematoma secondary to oral anticoagulant therapy intoxication reported in Serbia.

Gastrointestinal hemorrhage can be life-threatening due to frequent subclinical manifestations, possibilities of intramural and extra luminal bleeding, difficult endoscopic diagnosis in case of massive intraluminal and intramural bleeding and poor results of surgical treatment. Surgical

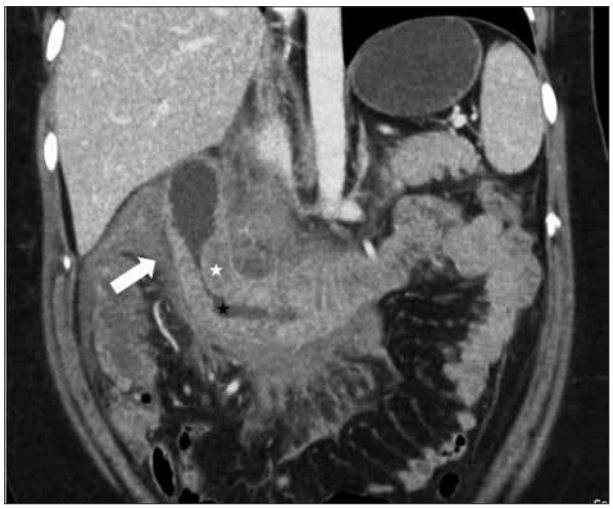

Figure 4. Contrast enhanced abdominal CT – multiplanar coronal reconstruction: thickened and predominantly hyperdense D2 and D3 duodenal wall (white star), with luminal narrowing, and hyperdense periduodenal mass (thick arrow), represents intramural and periduodenal hematoma

treatment should be considered when there is no evidence of partial resolution after conservative treatment, or in cases of perforation or peritonitis, which increases the size of the hematoma [13, 14].

Noninvasive imaging diagnostic tools such as abdominal US or CT scans may help in early diagnosis of intramural hematomas, as well as in detecting other locations of bleeding such as retroperitoneum and peritoneum, urinary tract, and soft tissues. US and CT also serve as imaging tools in evaluating the therapeutic effects of conservative and surgical treatment. Transabdominal ultrasonography is useful in detection of a thickened duodenal wall and distended fluid-filled stomach as an indirect sign of "gastric outlet" obstruction. CT is crucial in diagnosing intramural hematoma and its causes, and important in monitoring the effects of therapy [1, 2]. If duodenal hematoma is suspected, abdominal CT should be performed in both unenhanced and contrast-enhanced phases after the peroral preparation (the stomach and the duodenum distended with water) [15]. Typical CT presentation of intramural duodenal hematoma, like other intramural hematomas, includes circumferential ring-like hyper dense duodenal wall thickening with luminal narrowing in the native phase, without marked post-contrast enhancement in both arterial and venous phases [1, 2, 15, 16]. In addition to the intramural hematoma, duodenal perforation or intraluminal, periduodenal, and retroperitoneal active bleeding can be detected by contrast-enhanced CT [1, 2]. Due to a similar CT presentation, complicated duodenal ulcer (hemorrhagic or perforated), villous adenoma, or lymphoma of the duodenum could be considered in differential diagnosis with the intramural duodenal hematoma [1].

In summary, we described a rare complication of oral anticoagulant drug therapy, the spontaneous intramural duodenal hematoma. We concluded that intramural duodenal hematoma should be considered in patients presenting with symptoms of gastric outlet obstruction and gastrointestinal bleeding following the anticoagulant therapy. Ultrasonography and CT are useful diagnostic tools in detecting the hematoma, extramural and other locations of hemorrhage and in monitoring the therapeutic effects.

Conflict of interest: None declared.

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Спонтани интрамурални хематом дуоденума као последица интоксикације антикоагулантним лековима

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САЖЕТАК

Увод Хематоми дуоденума најчешће су трауматске етиологије, проузроковани ударном повредом трбуха. Нетрауматски спонтани интрамурални хематоми дуоденума су ретки и у највећем броју случајева узроковани антикоагулантном терапијом. Дијагноза се базира на клиничким и биохемијским параметрима, налазима ендоскопије и радиолошких метода прегледа.

Циљ овог рада је приказ клиничке слике и радиолошке презентације интрамуралног хематома дуоденума проузрокованог антикоагулантном терапијом.

Приказ болесника Жена стара 43 године се јавила лекару због епигастричног бола, мучнине, хематемезе и мелене. У личној анамнези је постојао податак о дубокој венској тромбози, која је дијагностикована месец дана раније, због чега је примала антикоагулантну терапију на бази кумарина. Физикалним прегледом је констатована дифузна болна осетљивост трбуха, а лабораторијским анализама повишен *INR* (преко 7), што је указивало на интоксикацију антикоагуланса. Компјутеризованом томографијом (КТ) приказано је сужење лумена, са равномерним, циркумферентним задебљањем зида десцендентног и хоризонталног дела дуоденума; зид је био хипердензан у нативној фази, са слабом и равномерном контрастном опацификацијом у виду прстена у постконтрастним фазама, што је типична презентација на КТ интрамуралног хематома дуоденума. Такође су визуализовани перидуоденални и десностран параренални хематом. После обустављања антикоагулантне терапије и укључивања конзервативне терапије (витамин К), са добрим терапијским одговором који је потврђен физикалним налазом, лабораторијским анализама и ултразвуком, на контролном прегледу КТ после две недеље констатована је спонтана резолуција интрамуралног хематома дуоденума.

Закључак Ултразвук и КТ су корисне дијагностичке методе у препознавању интрамуралног хематома дуоденума, екстрамуралне и других локализација крварења, као и у праћењу терапијског ефекта.

Кључне речи: хематом дуоденума; интоксикација антикоагулансима; ултразвук; компјутеризована томографија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Severe painful lower limbs and refusal of the leg reliance as atypical presentation of Gullain–Barre syndrome

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SUMMARY

Introduction Guillain–Barre syndrome (GBS) is the most common cause of acute flaccid paralysis in healthy infants and children. Acute motor axonal neuropathy (AMAN) is a type of GBS characterized by motor syndrome with no sensory symptoms.

Case outline Authors describe a six-and-a-half year old girl with atypical clinical presentation of AMAN with severe painful lower limbs and refusal of the leg reliance with typical findings on nerves conduction studies.

Conclusion Despite the nerve conduction study findings, atypical forms of AMAN and GBS are possible. Pain symptoms must be taken very seriously and treated careful by the clinicians.

Keywords: Guillain–Barre syndrome; acute motor axonal neuropathy; atypical presentation

INTRODUCTION

Guillain-Barre syndrome (GBS) is an acute, immune-mediated, demyelinating, peripheral neuropathy. GBS is the most common cause of acute flaccid paralysis in healthy infants and children [1]. Classic presentation and symptoms of GBS include ascending muscle weakness with sensory symptoms being a relatively minor feature and decreased or absent muscle tendon reflexes. GBS patients were divided into those with acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy and Miller Fisher syndrome [2]. The most frequent subtype of GBS in North America and Europe is AIDP, which accounts for up to 90% of GBS cases [3, 4]. AMAN is characterized clinically by nearly pure motor syndrome without sensory involvement and final diagnosis of AMAN is based on electrophysiological findings such as decreased amplitude of compound muscle action potential (CMAP) without any evidence of demyelination or change in sensory nerve action potential (SNAP) [5].

In this paper, we shall report on an atypical presentation of AMAN with severe pain in the lower limbs and refusal of the legs reliance with typical decreased amplitude of CMAP without any evidence of change in SNAP.

CASE REPORT

A six-and-a-half year old girl was admitted to the emergency department of the University Children's Hospital. She was somnolent with severe pain and muscle weakness in lower limbs, apthous ulcers in the mouth and prostration. The symptoms started seven days before admission with a high fever of 39°C and aphthous ulcers in the mouth. The next day, she complained about exhaustion, severe pain, and muscle weakness in lower limbs and she was unable to stand up and walk, so she was admitted to the regional hospital. The following day, her mother found her unconscious in bed, non-responding, with eyes and jaws fixed and livid lips with saliva leaking, so she was immediately transferred to the University Children's Hospital.

On admission to the emergency department of the University Children's Hospital, the girl was somnolent but responsive when called, afebrile, eupneic, acyanotic, and anicteric, pale and cold peripherally. Neurological findings included somnolent, disoriented. Beside dysarthria, other cranial nerves were intact. On upper limbs mild hypotonia, hyporeflexia and mild muscle weakness was presented. On lower limbs, severe muscle weakness and hypotonia were found with areflexia. Babinski sign was negative. Meningeal reactions were positive. Sensations on upper limbs were normal, on lower limbs with severe painful sensations, which were presented until the end of the hospitalization and beside the muscle weakness in the lower limbs, the most dominant clinical symptom. There was no loss of sensory stimuli or pins and-needles sensation. The intestine and bladder sphincters were intact. Autonomic functions were normal.

Personal and family history revealed no specific information. Neurological development

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was compatible with her age and all of immunizations were on schedule with no recent immunizations.

Blood tests revealed elevated C-reactive protein 42.5 and creatine kinase 257 U/L. Later on these parameters decreased. Analysis of the cerebrospinal fluid revealed mild protein content (0.648 g/L), 20 leukocytes, and glucose was 3.7 mmol/L. The serologic tests were negative for the herpes virus, rubella, Epstein–Barr virus, rubeola, toxoplasmosis, cytomegalovirus, enteroviruses, respiratory viruses, Lyme disease, and *Mycoplasma pneumoniae*. There were no microbial proliferations in the culture for *Salmonella*, *Shigella* and *Campylobacter jejuni*. Computed tomography scan of the endocranium was within the normal range. The child underwent nerve conduction studies (NCS). The findings of motor NCS in the lower limbs showed reduced amplitude of CMAPs, whereas distal latencies and motor conduction velocities were normal. The findings in the upper limbs showed also reduced amplitudes, whereas distal latencies and motor conduction velocities were normal. Findings of sensory NCS were interpreted as within normative ranges with normal values for SNAP, latencies and velocities (Table 1).

Table 1. Nerve conduction studies values for tested nerves

Type of nerve	Name of nerve	R/L ms (Normal values) [6]	R/L mV (Normal values) [6]	R/L m/s (Normal values) [6]
		Distal latency	CMAP	NCS
Motor	Median	2.85/2.8 (≤ 4.4)	2.8/3 (≥ 4)	55/58 (≥ 49)
	Ulnar	2/2.1 (≤ 3.3)	4.5/4.7 (≥ 6)	62/60 (≥ 49)
	Peroneal	2/1.8 (≤ 6.5)	1.1/1 (≥ 2)	55.1/55.7 (≥ 44)
	Tibial	3.15/3.2 (≤ 5.8)	1.2/1 (≥ 4)	54.3/50.3 (≥ 41)
		Latency	SNAP	NCS
Sensory	Sural	1.7/1.7 (≤ 4.4)	12.9/14 (≥ 6)	50.1/47.7 (≥ 40)

CMAP – compound muscle action potential; NCS – nerve conduction studies; SNAP – sensory nerve action potential

Based on clinical features with motor involvement, electrophysiological investigation, as well as the normal laboratory findings, this patient was diagnosed with AMAN subtype of GBS. Findings of sensory NCSs were normal in spite of the severe pain in the lower limbs.

The patient was treated with intravenous immunoglobulin and general supportive therapy. She did not require respiratory support. Rehabilitation treatment started early, right after the stabilization of the general condition. Muscle strength of the lower and upper limbs started to improve immediately after medication, but pain did not decrease with the introduction of Carbamazepine, so she required opioids for pain management in the beginning. After five weeks, muscle strength on the upper limbs was grade five and muscle strength on lower limbs had improved to grade four, so she could stand independently, but severe pain in the lower limbs persisted and the child refused to stand up and walk. Eight weeks after admission, muscle strength on lower limbs improved to grade five and painful sensations decreased so she could stand up and walk independently. Three months later, at the outpatients' follow-up, she had fully recovered.

DISCUSSION

Guillain-Barre syndrome (GBS) is an acute, immune-mediated, demyelinating, peripheral neuropathy. The disease is thought to be autoimmune and triggered by a preceding infection in two thirds of cases, most frequently respiratory or gastrointestinal infections [7, 8].

Campylobacter jejuni infection has been associated with GBS. GBS that occurs after *Campylobacter jejuni* infection is usually more severe related with extensive axonal injury [9]. The following infections have also been associated with GBS: cytomegalovirus, influenza, *mycoplasma pneumoniae*, Epstein–Barr virus infection, or mononucleosis, HIV or AIDS. The incidence of GBS after immunization was not different from the background incidence of GBS, thereby precluding any firm conclusions about the significance of these findings. However, because of the close temporal association of GBS with selected vaccines, the risks and benefits of immunization merit individual review by the clinician and patient [3]. The incidence of GBS is between 1.1/100,000/year and 1.8/100,000/year with lower rates reported in children (< 16 years) of around 0.6/100,000/year. The age of onset of GBS in our patient was in accordance with previous reports [4]. The review reported mostly on studies from Europe and North America [8]. As it was previously stated that the frequency of AIDP subtype of GBS is high in US and Western Europe, it should be stated as well that the AMAN is frequent (up to 65%) in the study conducted in China [4]. NCS and cerebrospinal fluid analysis is important investigations that help confirm the diagnosis of GBS [5]. Although GBS is a relatively uncommon condition, the consequences of a missed or delayed diagnosis and delayed treatment can lead to progression of muscle weakness and a worse outcome [10]. Our findings on NCS correlated with classical patterns for AMAN [11]. Treatment of GBS patients requires a multidisciplinary approach [3, 12]. Based on clinical features with motor involvement, electrophysiological investigation, as well as the normal laboratory findings, this patient was diagnosed with AMAN subtype of GBS with severe painful legs as atypical presentation of this condition. Lee and Han [13] as well as Neocleous et al. [14] reported the first case of AMAN confirmed by electrophysiological studies that was accompanied by severe pain of the entire body. In our patient, at the beginning, severe pain sensations were treated with opioids and Carbamazepine. Later on, opioids were excluded and painful sensations were successfully treated only by Carbamazepine. Muscle strength was the first to return, but pain persisted after full muscle recovery. Three months later, at the outpatients' follow-up, she had fully recovered.

Despite the NCS findings, atypical forms of AMAN and GBS are possible. Painful symptoms must be taken very seriously and treated carefully by the clinicians.

Conflict of interest: None declared.

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Јак бол у доњим екстремитетима и одбијање вертикализације као атипична презентација Гилен–Бареовог синдрома

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САЖЕТАК

Увод Гилен–Бареов синдром (ГБС) представља најчешћи узрок акутне флакцидне парализе код здраве одојчади и деце. Акутна моторна аксонална неуропатија (АМАН) представља тип ГБС-а и карактерише се моторним синдромом без сензорних симптома.

Приказ болесника Аутори приказују шестоипогодишњу девојчицу са атипичном клиничком презентацијом АМАН-а. У налазу доминира јак бол у доњим екстремитетима и одбијање вертикализације са типичним електронеурографским налазом.

Закључак Иако електронеурографски налаз показује типичне знаке ГБС-а, клинички су могуће појаве атипичних форми АМАН-а у склопу ГБС-а. Болни симптоми се морају правовремено препознати и адекватно клинички третирати.

Кључне речи: Гилен–Бареов синдром; акутна моторна аксонална неуропатија; атипична презентација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Left ventricular assist device implantation and concomitant aortic valve replacement

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SUMMARY

Introduction The implantable device for mechanical support of the left ventricular circulation (LVAD) is widely applied as a therapeutic option for survival and improvement of the quality of life in patients with the end-stage heart failure.

The objective of our paper was to present the implantation of the aforementioned device together with the aortic valve replacement in the same procedure.

Case outline The patient was admitted to the hospital during his terminal stage of heart failure, with ejection fraction of 18%. The ergospirometry test showed that the maximum VO_2 was 10.1 ml/kg/min. Because the medicament therapy hadn't provided adequate results, the LVAD device was implanted as a bridge until transplantation. Due to severe aortic insufficiency, the aortic valve was concomitantly replaced with bioprosthesis in order to prevent the negative effect of this valvular disease on pump work and clinical outcome.

Conclusion This case report confirms that LVAD implantation with the correction of a significant aortic insufficiency is a procedure with satisfactory short-term and long-term results.

Keywords: cardiac failure; LVAD; aortic valve

INTRODUCTION

The implantation of the left ventricular assist device is a therapeutic option for the treatment of end-stage heart failure patients. However, this group of patients often suffers from different associated pathological changes of the heart, most commonly cardiac valves. Some of these defected valves require surgical correction at the same time when LVAD is being implanted. If not, they could interfere with the function of the device and have unfavorable effect on the clinical outcome [1].

In addition, uncorrected aortic insufficiency (AI) at the time of LVAD implantation may progress and affect the effectiveness of the pump by limiting forward flow [2].

We present the first case report in Serbia of the implantation of the LVAD and concomitant aortic valve replacement in patients with the end-stage heart failure.

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CASE REPORT

A 64-year-old male patient presented in the end-stage heart failure due to ischemic cardiomyopathy. The patient mentioned fatigue and continuous squeezing chest pains as symptoms. He had also been treated for bronchial asthma and frequent respiratory infections. In the previous two years, the patient had been hospitalized four times due to heart failure symptoms. Selective coronarography showed that left anterior descending artery had a proximal stenosis around 90–95%, while the circumflex artery was occluded in its medial segment. The proximal part of the right coronary artery was also occluded.

Ergospirometry (cardiopulmonary exercise testing) showed reduced exercise capacity with peak oxygen consumption (VO_2 peak) 10.1 ml/kg/min.

Single-photon emission computerized tomography showed the absence of viable myocardium of the apex, lateral, and inferior walls.

Echocardiography recorded severely impaired ejection fraction (EF) of the left ventricle with combined aortic defect. The complete aortic defect manifesting with aortic stenosis and low flow gradient due to extremely impaired systolic function of the left ventricle was evident (aortic valve area was 1.1 cm^2, peek gradient was 27, V max 2,6). AI of 2–3+ was recorded. The left ventricle dimensions were enlarged, end-diastolic diameter (EDD) was 7.2 cm, end-systolic diameter (ESD) was 6.6 cm with EF of 20% by Biplane and 18% by Teicholz. Echocardiography also recorded akinetic septum and basal segment of the anterior wall, akinetic posterior wall, as well as fibrously modified and dyskinetic basal inferior wall. Mitral valve morphology was preserved. The left moderate to severe atrial mitral regurgitation of 2–3+ with its normal dimensions, i.e. 3.9 cm, was noted. The right ventricular dimension was normal (1.8 cm), with good systolic and longitudinal functions, fractional area change

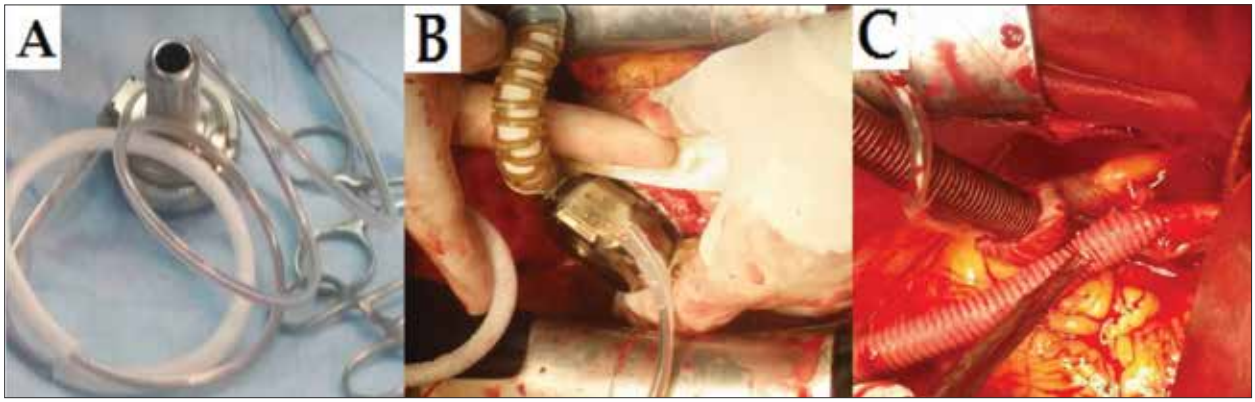

Figure 1. A) Preparation of the Heart Ware device – connecting the outflow graft to the pump and rinsing the pump; B) after the circular opening of the left ventricle and fastening of the ring, the pump was fixed and hemostasis was checked; C) outlet graft fastened to the ascendant aorta

of 50% and (tricuspid annular plane systolic excursion) TAPSE of 24 mm.

The patient was categorized as New York Heart Association (NYHA) class IV, Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) class 4.

Upon the complete preoperative preparation, the patient was operated on in the conditions of extracorporeal circulation. After median sternotomy, the patient was heparinized and cannulated. Aortic valve replacements preceded pump implantation. Myocardial protection was achieved using antegrade cardioplegia solution. The aortic valve was replaced with a St. Jude Medical Biocor Bioprosthesis (Number 23).

The aortotomy was closed. After releasing the clamp, HeartWare LVAD (Medtronic, Minneapolis, MN, USA) was implanted on the beating heart. The inflow cannula device was installed over the top of the left ventricle, the output graft was connected with the ascending aorta, while the power cable was drawn through the skin (Figure 1).

The patient became fully activated in the postoperative period. The patient and his family members were educated on hygiene maintenance of the spot where the power cable exits the skin as well as interpretation of basic findings and LVAD controller alarm.

Echocardiography finding on discharge showed biological artificial aortic valve closing with each cardiac cycle. The left ventricle had mildly enlarged dimensions, EDD 5.8 cm and ESD 5 cm, and EF in basal segment was estimated to 29%. The right ventricle had normal dimension – 2.6 cm, good systolic and poorer longitudinal function, TAPSE 12 mm (underestimated due to the opening of the pericardium).

The pump speed was set at 2,600 rpm, pump flow at 6.7 l/min., pump power at 4.1 W, and spare controller at 2,600 rpm.

The therapy prescribed on discharge included the following: warfarin (according to therapeutic protocol so that international normalized ratio would be 2–3); acid acetylsalicylic 100 mg, ramipril tab. 2 × 5 mg, bisoprolol fumarate 1 × 5 mg, amiodarone 200 mg, furosemide tab. 1 × 20 mg, spironolactone 1 × 25 mg, trimetazidine 2 × 35 mg, pantoprazole 2 × 20 mg, and atorvastatin 1 × 10 mg.

On 30-day, two-month, six-month, and one-year control visits, the patient did not manifest the signs of heart failure, and LVAD parameters on the controller were stable.

The pump speed was set at 2,700 rpm, in order to achieve better unloading of the LV, with pump flow of 5 l/min and pump power of 4.4 W. Echocardiography examination at 15 months showed biological artificial aortic valve closing with each cardiac cycle, with normal flow gradients, improvement in EDD and ESD from baseline values of 7.2 cm and 6.6 cm to 6.4 cm and 4.9 cm, respectively and mild mitral regurgitation. For EF and B-type natriuretic peptide (pg/ml), the baseline values of 20% and 960, improved to 46% and 176, respectively. The dimension of the right ventricle was sustained in the normal range (2.6 cm) with good systolic function. There was normal flow through both inflow and outflow cannula.

DISCUSSION

The prevalence of a heart failure is roughly around 1–2% in adults and goes all the way up to 10% in patients older than 70 years [3].

The therapy of choice for treating end-stage heart failure is the heart transplantation. However, the insufficient number of donors has accelerated the development of mechanical circulatory support (MCS) devices. In the last couple of decades, the biggest improvement (leap) in the treatment of heart failure was made in the usage of short-term MCS for cardiogenic shock, and long-term MCS for destination or bridge-to-transplant therapy [4].

Current indications for LVAD implantation are bridge-to-transplant patients, implantation as a permanent or destination therapy and a bridge to recovery of the heart's function in cases when there is a significant improvement of the heart's structure and function that is enough to achieve long-term disappearance of symptoms (in these cases, the explanation of the device is considered) [5].

The number of LVADs that are implanted worldwide is continuously rising. The growing experience of LVAD implantation has led to a substantial improvement of the outcome, with one-year survival rates approaching those

in patients with heart transplantation. These refinements have caused growing interest for expanding the clinical indications for LVAD therapy, especially in patients with less advanced heart failure [6, 7].

The criteria for LVAD implantation are NYHA class IV heart failure refractory to optimal medical therapy, left ventricular EF less than 25%, systolic blood pressure < 80 mmHg, pulmonary capillary wedge pressure > 20 mmHg, cardiac index < 2.0 l/min/m^2 despite continuous intravenous inotropic therapy and intra-aortic counterpulsation. In addition to these criteria, malignant cardiac arrhythmias, as well as patients who are on the transplantation waiting list can also be considered for the LVAD therapy. Patients who suffer from an advanced congestive heart failure are a bigger challenge and, therefore, physicians must monitor the symptoms closely in order to identify the right timing for the implantation of the LVAD. If the LVAD is implanted too early, benefits and the potential of this medical treatment to recover heart function will not be fully utilized. If the LVAD is implanted too late, the outcome may worsen due to a secondary organ damage caused by a prolonged heart failure.

It is important to note that valvular heart disease is often present. The decision to surgically manage valvular disease at the same time as LVAD implantation depends on several factors such as the influence of valvular disease on post-implantation period and indications for surgical management of a valvular disease [8].

It is known that AI is a complication in approximately 25% of patients with a non-pulsatile MCS device. Although the increase in LVAD speed improves hemodynamics, it also deteriorates aortic regurgitation (AR). AI in patients with LVAD support contributes to higher baseline central venous pressure, peek capillary wedge pressure, and lower pulmonary artery pulsatility index. [9].

Mitral stenosis must be managed during LVAD implantation, since the presence of the mitral valve prosthesis (biological or mechanical) is not a contraindication for LVAD implantation [10].

Secondary tricuspid regurgitation (TR) is frequent in patients with the associated failure of the right cardiac ventricle who are undergoing a LVAD implantation. The decision to perform a tricuspid valve repair during LVAD implantation is in correlation with moderate-to-severe degree of TR. If TR was corrected, it might have benefit on venous flow and renal perfusion and also improve postoperative morbidity [11].

Truby et al. [12] reported that out of 10,603 eligible patients, 1,399 patients on CF-LVAD support developed moderate to severe AI. The prevalence of a significant AI progressively increased over time. The predictors of AI worsening included older age, female sex, smaller body mass index, mild pre-implantation AI, and destination therapy strategy. Moderate to severe AI was associated with significantly higher left ventricular EDD, reduced cardiac output, and higher levels of brain natriuretic peptide. Significant AI was associated with higher rates of rehospitalization (32.1% *vs*. 26.6%, respectively, at two years; p = 0.015) and mortality (77.2% *vs.* 71.4%, respectively, at two years; p = 0.005), conditional upon survival to one year. [12, 9].

The surgical strategy and timing of significant AR surgical management have not been fully defined. There have been several articles describing a few treatments of AR at the time of LVAD implantation. Understanding of the AI after MCS is evolving; however, continuous closure of the aortic valve is thought to be the main cause. Careful attention to outflow cannula orientation in order to prevent direct flow towards the aortic valve can minimize the stress on the valve [9].

Today, the most common procedure is a simultaneous aortic valve replacement with bioprosthesis. However, you may also find reports of patch closure of the outflow tract, primary aortic cusp closure with felt strips, and coaptation stitching of the valve cusps that are more rare procedures [13].

The bioprosthetic valve replacement has the advantage of eliminating valve pathology altogether and not rendering the patient LVAD-dependent. It is very important to know that the controlled work pump and heart beat ratio provide occasional opening of the aortic valve (or bioprosthesis) that could potentially prevent the development of clot formations and fusion of the aortic root washout [14].

Timing of the aortic valve replacement is a unique clinical challenge as well, and the decision is made based on the degree of AR, as well as indications for LVAD implantation. Patients with mild to moderate AR who belong to the "bridge-to transplant" group, where a shorter time of organ donation is expected, the replacement of aortic valve is not necessary. On the other hand, in the "destination therapy" group and in patients with significant AR, aortic valve replacement during LVAD implantation is a reasonable option [15].

The case presented in our report underwent implantation of LVAD for maintaining vital parameters and eliminating the symptoms of heart failure. The significant aortic failure was repaired simultaneously with LVAD implantation by replacing the impaired valve with bioprosthesis. This case report shows that LVAD implantation, along with correction of significant AI by replacing the aortic valve with bioprosthesis, is a procedure that has satisfying results.

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.

Conflict of interest: None declared.

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Имплантација уређаја за механичку циркулторну потпору леве коморе и придружена замена аортне валвуле

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САЖЕТАК

Увод Имплантабилни уређаји за механичку потпору циркулације леве коморе (*LVAD*) широко се користе као терапијска опција за преживљавање и побољшање квалитета живота болесника са терминалном срчаном слабошћу.

Циљ рада је презентација имплантације уређаја за трајну механичку циркулаторну потпору леве коморе уз замену аортне валвуле у истој процедури.

Приказ болесника Болесник је хоспитализован у терминалном стадијуму срчане инсуфицијенције са ејекционом фракцијом од 18%. Ергоспирометријски тест је показао максимум VO_2 од 10,1 *ml/kg/min*. С обзиром на то да медикаментозна терапија није дала задовољавајуће резултате, уграђен је *LVAD* као мост до трансплантације срца. Због значајне аортне инсуфицијенције валвула је замењена биопротезом да би се спречио неповољни утицај на рад пумпе и клинички исход.

Закључак Имплантација *LVAD*-а уз корекцију значајне аортне инсуфицијенције је процедура са задовољавајућим краткорочним и дугорочним резултатима.

Кључне речи: срчана слабост; *LVAD*; аортна валвула

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Vertebral erosion due to chronic rupture of aneurismatic abdominal aorta

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SUMMARY
Introduction Extremely rarely, the evolution of abdominal aortic aneurysm (AAA) includes the phase when extravasations of the blood from a ruptured aneurysm is contained by the surrounding tissue, referred to as chronic (contained) rupture of the AAA.
Our aim was to call attention to this life-threatening condition, which is always challenging for diagnosis.
Case outline A 58-year-old man reported to the Emergency Center for significant abdominal pain. Ultrasound examination showed an infrarenal aneurysm of the abdominal aorta. A computed tomography scan of the thorax, abdomen, and pelvis with iodine contrast in arterial phase was performed. A free gas collection was observed between the liver and the anterior abdominal wall that is traced to a ruptured inflamed diverticulum on the transversal colon. Immediately distal to the branching sites of the renal arteries, the abdominal aorta extended forward and aneurismatically expanded. Posterior left, along the psoas muscle, a rupture of the aortic wall was seen, with an organized hematoma that accompanied the muscle. Between the hematoma and the aortic aneurysm, erosions of the anterior and lateral part of the vertebral bodies L2 and L3 were discovered. The patient underwent endovascular AAA repair (EVAR) and recovered well.
Conclusion Multidetector computed tomography angiography is a reliable, non-invasive, and necessary examination for localization and evaluation of the size of the AAA form, its chronic rupture, and complications such as vertebral body erosion.
Keywords: abdominal aorta aneurysm; chronic rupture; vertebral body erosion

INTRODUCTION

Abdominal aortic aneurysm (AAA) is a dilatation of its wall up to a diameter greater than 30 mm. AAA rupture is a significant cause of death for people over 55 years of age [1]. In cases where after a rupture and under certain circumstances hematoma formation occurs with localized and partly organized bleeding, a chronic AAA rupture is created which occurs in only 4% of all AAA cases [2]. One of the few complications can be the usurpation of vertebral bodies as a result of long-term compression on them [3].

CASE REPORT

A 58-year-old man reported to the Emergency Center for significant abdominal pain. There wasn't any history of diabetes, hypertension and chronic lung disease. He has been smoking and had myocardial infarction 15 years ago and had duodenal ulcer surgery 10 years ago. Physical examination revealed that the patient was sub-febrile and normotensive, and palpatory examination of the abdomen revealed pulsations in supra and umbilical region. Laboratory findings were within normal limits. Ultrasound examination showed an infrarenal aneurysm of the abdominal aorta. In the native abdominal image in the supine position, we noticed a smaller free gas collection – pneumoperitoneum. A computed tomography (CT) scan of the thorax, abdomen, and pelvis with iodine contrast in the arterial phase was performed. We found right pleural effusion diameter of 25 mm, with no active pathological changes in the lungs. There was no significant finding in the mediastinum. A free gas collection was observed between the liver and the anterior abdominal wall, traced to a ruptured inflamed diverticulum on the transversal colon. Perihepatic and perisplenic free fluid and signs of mesenteritis were found. A ventral hernia of the anterior abdominal wall with bowel and adipose tissue within the hernia sac was noted. Immediately distal to the branching sites of the renal arteries, the abdominal aorta extended forward and aneurismatically expanded the largest diameter of about 15 cm, calcified wall with a marginal thrombus mass of about 16 cm in length (Figure 1). Posterior left, along the psoas muscle, a rupture of the aortic wall was

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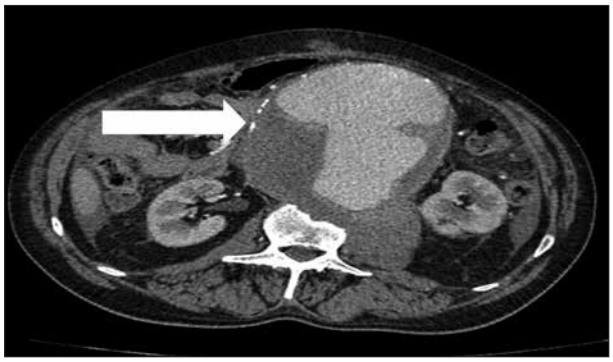

Figure 1. Computed tomography aortography showing the abdominal aorta extending forward and aneurismatically expanding with calcified walls and a marginal thrombus mass (white arrow)

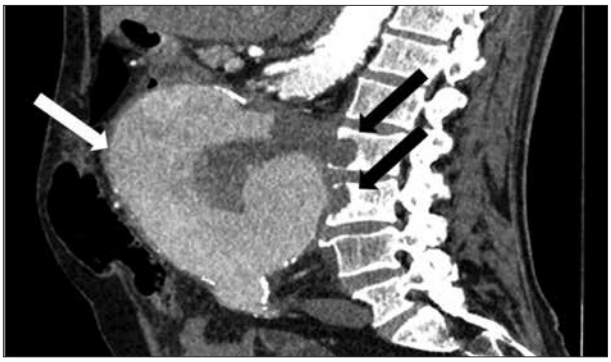

Figure 2. Sagittal computed tomography reconstruction showing the aortic aneurysm (white arrow) and discovered erosions of the anterior and lateral parts of the vertebral bodies L2 and L3 (black arrows)

seen, with an organized hematoma that accompanied the muscle about 12 cm in length. Between the hematoma and the aortic aneurysm, erosions of anterior and lateral parts of the vertebral bodies L2 and L3 were discovered (Figure 2). Right common iliac artery was aneurismatically dilated up to 35 mm in diameter, while the left one was of normal lumen width, with both having calcified walls.

The patient underwent endovascular AAA repair (EVAR), and he recovered well.

Before the EVAR procedure, the ruptured diverticulum needed to be resolved. After drainage of the peritoneum, surgeons performed colonic resection with primary anastomosis without colostomy placement. For the next 10 days, the patient was on antibiotic therapy. Postprocedural complications did not occur.

DISCUSSION

Chronic AAA rupture is a rare complication that occurs in 4–7% of cases [2, 4]. On CT examination, it appears as a sign of a "wrapped" aorta. It is presented as the discontinuity of the calcified aortic wall, a clearly limited mass of soft tissue density vaguely restricted from other adjacent structures (spine, psoas muscle) [5, 6]. There are no signs of contrast extravasation within the mass described, which clearly differentiates it from true AAA rupture [7, 8, 9]. The diagnosis of chronic AAA rupture is very important and necessary in order to be aware of possible complications, such as the erosion of vertebral bodies, as well as to find out their causes. The complications of vertebral bodies' erosion could be paraplegia, inflammation, as well as death [10, 11].

Destruction of vertebral bodies occur as a complication in only 7% of AAA cases [3]. The cause of erosion of vertebral bodies can be a wide range of diseases of different etiology: metastases, vertebral tumors, vertebral fractures, osteoporosis, and spondylitis [12, 13, 14]. They can occur separately from AAA in inflammatory diseases such as Behcet's disease and syphilis. Compressive uses can occur in retroperitoneal tumors and retroperitoneal abscesses, and this is where CT diagnosis is crucial in differentiating from the compressive effect of chronic AAA rupture [6].

In the case of our patient, we noticeD the sign of a "wrapped" aorta as the discontinuity of the calcified wall, then the mass of soft tissue density (formed by the old hematoma) extending from the AAA, vaguely delimited by the left psoas muscle and in contact with the spinal column and usurpations of the L2 and L3 vertebrae. We consulted an orthopedist and decided not to do anything with vertebral bodies and to let them repair spontaneously. Our patients underwent EVAR, the gold standard for repairing aneurismatic dilatation of the aorta [14, 15, 16]. The patient recovered well. Ten days after the procedure, he was discharged from the hospital, and has regularly been reporting for check-ups, without any sign of complication.

Based on the literature and as presented in our case, we can conclude that multidetector computed tomography angiography is a reliable non-invasive and necessary examination for localization and evaluation of the size of the AAA form, as well as for differential diagnosis of its complications.

Conflict of interest: None declared. The report follows the ethical guidelines of the most recent Declaration of Helsinki (Edinburgh, 2000) and has received approval from the local ethics committee.

Informed consent statement: Consent was obtained from the patient for publication of this report and any accompanying images.

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Ерозије на вертебралним пршљенским телима услед хроничне руптуре анеуризме абдоминалне артерије

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САЖЕТАК

Увод Изразито ретко се код анеуризме абдоминалне аорте може видети фаза када се екстравазација крви из руптуриране анеуризме задржава у околном ткиву, што се назива хроничном (ограниченом) руптуром анеуризме абдоминалне аорте.

Циљ рада је био да се скрене пажња на ово животно угрожавајуће стање, које није увек лако открити.

Приказ болесника У Ургентни центар се јавио 58 година стар мушкарац због значајног бола у стомаку. Ултразвучно је верификована анеуризма инфрареналног дела абдоминалне аорте. Урађена је компјутеризована томографија са јодним контрастом. Откривена је колекција гаса између јетре и предњег трбушног зида која је праћена до руптурираног инфламираног дивертикулума трансверзалног дела колона. Непосредно испод исходишта реналних артерија уочило се анеуризматско проширење аорте које се пружало унапред. Позади лево, дуж *m. ilipsoasa*, уочена је руптура зида аорте са организованим хематомом који се пружа унутар мишића. Између хематома и анеуризматског проширења се виде ерозије предњег и спољашњег дела пршљенских тела *L*2 и *L*3. Урађена је ендоваскуларна репарација анеуризме абдоминалне аорте и болесник се добро опоравио.

Закључак Компјутеризована томографска аортографија, иначе неинвазивна метода, показала се као поуздана и неопходна у случају откривања хроничне руптуре анеуризме абдоминалне аорте са компликацијом у виду ерозија кичмених пршљенова.

Кључне речи: анеуризма абдоминалне аорте, хронична руптура, ерозије пршљенских тела

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Laparoscopic splenectomy in the treatment of splenic artery aneurysm – case report and literature review

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SUMMARY

Introduction Splenic artery aneurysm is the most common visceral aneurysm with a prevalence of 0.2–10%. It is the third most frequent abdominal aneurysm as well. It can be true or false. It occurs more often in women than in men. We present our experience with a 34-year-old female patient who underwent laparoscopic splenectomy due to the splenic aneurysm located in the splenic hilum.

Case outline We present a case of a 34-year-old female patient diagnosed with an enlarged splenic artery during a routine abdominal ultrasound examination. Abdominal scan and computed tomography angiography showed saccular aneurysm of the splenic artery located in the hilum of the spleen, 24 × 17 mm in size. Given the good general condition and age of the patient, we decided to perform laparoscopic splenectomy. The operation was performed without complications, which was also the case with the postoperative flow. The patient was discharged from the hospital on the third postoperative day.

Conclusion Laparoscopic splenectomy is a safe and effective modality for the treatment of splenic artery aneurysm, localized in the splenic hilum. Considering all the benefits of minimally invasive surgery, laparoscopic splenectomy should be the treatment of choice, over the classical open approach.

Keywords: spleen; aneurysm; splenic artery; laparoscopic splenectomy

INTRODUCTION

Splenic artery aneurysm (SAA) is the most common visceral aneurysm with a prevalence of 0.2–10%. It is the third most frequent abdominal aneurysm [1]. According to standard classification criteria SAA can be true (involves all three layers of an artery) or false, i.e. pseudoaneurysm (collection of blood that forms between the two outer layers of an artery). It occurs more often in women than in men, by a ratio of 4:1. Most aneurysms are less than 2 cm in diameter, saccular and commonly found at the center or the distal part of the splenic artery [1, 2].

The main risk factors for lienal artery aneurysm that have been identified are female sex, fibromuscular dysplasia, vascular diseases, multiple pregnancies, and portal hypertension [2, 3]. Abdominal ultrasound or Doppler ultrasonography, computed tomography (CT), nuclear magnetic resonance (NMR), selective and CT angiography are used to diagnose this disease [4]. Aneurysms with a diameter larger than 2 cm require surgical treatment, as well as symptomatic aneurysms less than 2 cm in diameter. Aneurysms less than 2 cm and without symptomatology can be radiologically monitored [1, 4, 5].

There are several treatment modalities for SAAs: open resection, splenectomy, as well as endovascular treatment (graft, stent, or embolization), depending on the patient's suitability for a particular type of treatment [6, 7].

In this paper, we present laparoscopic splenectomy as a safe and effective treatment modality for SAA located in the splenic hilum.

CASE REPORT

A 34-year-old female patient diagnosed with an enlarged splenic artery in routine abdominal ultrasound examination was admitted to the Clinic for Digestive Surgery within the Clinical Center of Serbia on March 15, 2019. By examining her medical documentation, we found out that the patient was being treated for hypertension with an ACE inhibitor. All laboratory findings on admission were within normal range with a body mass index of 31.67 kg/m^2. Abdominal CT scan and CT angiography verified enlarged, tortuous splenic artery and saccular aneurysm in the splenic hilum, 24 × 17 mm in size, with intimal calcification and thrombus (Figure 1).

Considering radiological findings, we decided to perform splenectomy. Because the patient was a young woman in good medical condition, we opted for a laparoscopic approach.

To prevent the formation of thromboembolic complications, the patient was preoperatively

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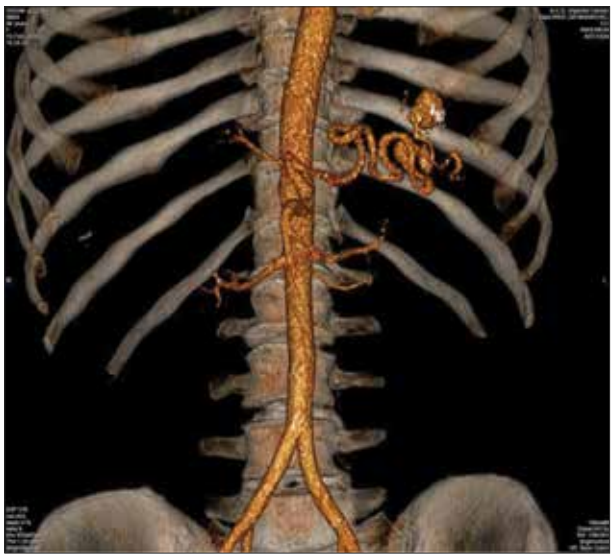

Figure 1. Multidetector computed tomography angiography showed splenic artery aneurysm in the splenic hilum

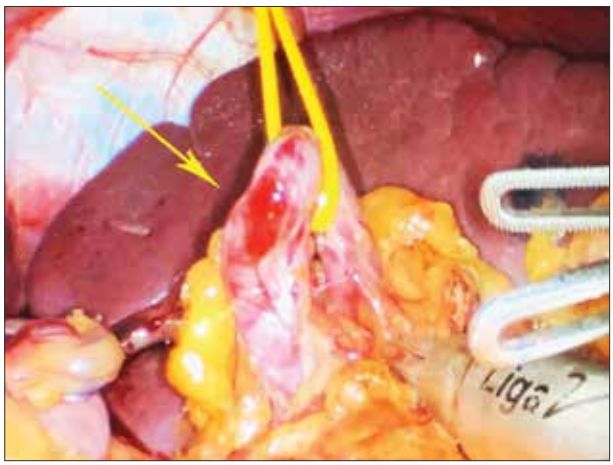

Figure 2. Intraoperative photo: splenic artery aneurysm pulled with a yellow rubber band

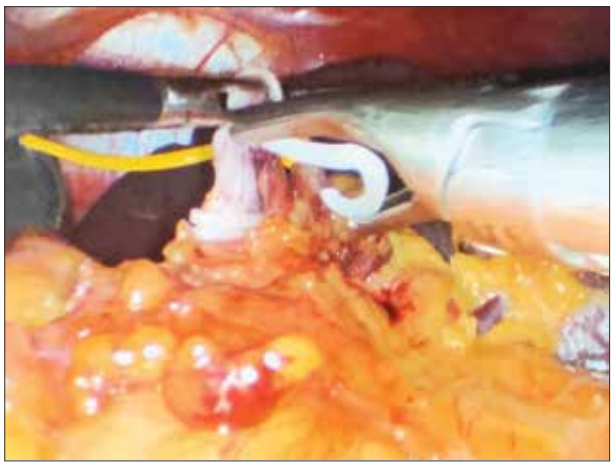

Figure 3. Intraoperative photo: key step for aneurysm clipping with "hem-o-lok" clips

Figure 4. Removal of the spleen with an endobag

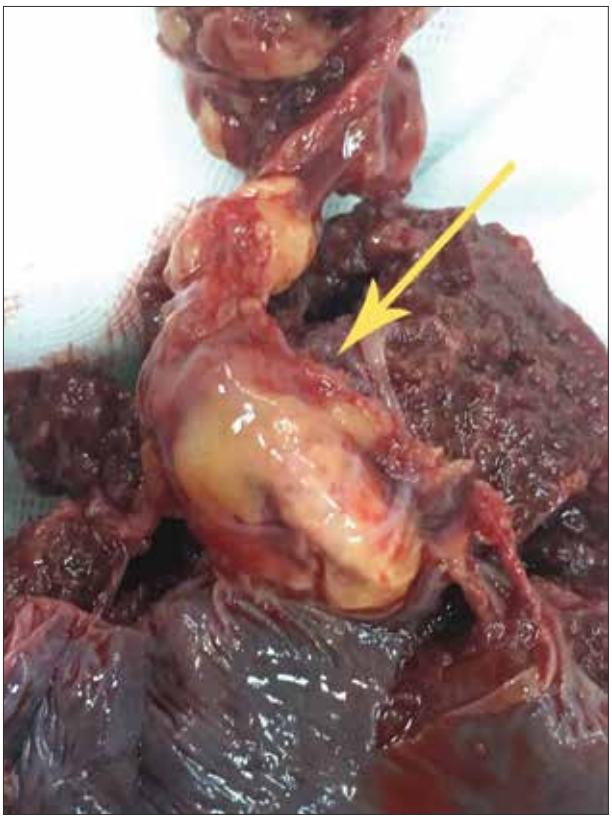

Figure 5. Operative specimen with splenic artery aneurysm

treated with low-molecular-weight heparin. After induction of general endotracheal anesthesia, the patient was positioned in the right hemilateral position, i.e. the "hanging or leaning spleen" technique [8]. After port placement and insertion of a laparoscope, the examination of the abdomen confirmed the preoperative finding. An aneurysm of the splenic artery in the hilum of the spleen was identified. We first mobilized the spleen by cutting the splenic ligaments and short gastric vessels with a laparoscopic harmonic scalpel. After complete mobilization of the spleen, we started the preparation of aneurysm and its separation from the surrounding structures (Figure 2). After preparation of the artery, a few centimeters proximally from the aneurysm, we placed two hem-o-lok clips proximally and one distally, and then cut the artery (Figure 3). The splenic vein was treated the same way. After taking care of the elements of the hilum, the spleen was completely separated from the surrounding structures, placed in the endobag

(Figure 4), and thus removed from the abdomen. Hemostasis was checked and the abdominal tube was placed. The splenic specimen was sent to the histopathological examination (Figure 5).

A definitive histopathological finding showed that the tissue of the spleen had preserved histomorphology and that the splenic artery had the aneurysmatic expansion, sclerosis and focal calcification.

There were no postoperative complications. The nasogastric tube was removed on the first and the abdominal tube on the second postoperative day. The patient was discharged from the hospital on the third postoperative day with prescribed antibiotic prophylaxis and postoperative immunization, according to the current literature and guidelines for the prevention and treatment of postsplenectomy complications [9, 10].

The 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.

DISCUSSION

St. Leger Brockman reported the first surgical case of an SAA in 1930 [1]. Saw et al. [11] performed the first laparoscopic-assisted SAA operation in 1993. SAA is the third most common type of abdominal aneurysm that accounts for 60% of all visceral aneurysms with a prevalence of 0.8% in the adult population. SAA is defined as a segmental enlargement of the artery with a diameter of 10 mm. SAA rupture is a life-threatening condition with a mortality rate of up to 75% [1, 12].

Most SAAs are true aneurysms, with higher representation in women. The main risk factors are female sex, atherosclerosis, arterial hypertension, multiple pregnancies [2]. According to the literature data, 10% of gigantic SAAs (> 5 cm) are associated with liver cirrhosis. Around 2.5% of patients have portal hypertension [2, 6, 13]. Pancreatitis is reported as the main risk factor for the emergency laparotomy to treat sudden rupture and bleeding from splenic artery pseudoaneurysms [6]. Pancreatic enzymatic autodigestion can cause weakening of the splenic artery wall architecture leading to pseudoaneurysm formation.

In most cases, SAA is asymptomatic. Most of them are discovered on routine examinations or as an incidental finding during the radiological imaging performed for another medical condition [2]. SAA can be diagnosed by abdominal ultrasonography, CT, NMR, and CT angiography [1, 4, 5].

In symptomatic patients, the most common complaints are epigastric or back pain. Some authors consider that all symptomatic patients, as well as patients with no symptoms, whose SAA is > 20 mm in diameter, should be surgically treated because of the possibility of rupture [12]. Particularly risky groups of patients are pregnant women, patients with portal hypertension, and patients in whom liver transplantation is planned [2, 12]. In patients without symptoms, in whom SAA is < 20 mm in diameter, radiological follow-up by abdominal CT every six months should be enough [1, 2, 12]. In our case, the patient was without symptoms. Because of the SAA of 24 mm in size, we opted for surgical treatment.

The modality of the SAA management is an open spleen-preserving aneurysm resection with splenic artery end-to-end anastomosis. Open or laparoscopic splenectomy is the treatment choice for aneurysms located in the splenic hilum or immediately next to the hilum. Another option is endovascular management with stent placement or arterial embolization [6, 7, 14, 15]. The aim of surgical treatment should be the treatment of aneurysm with the splenic preservation or the preservation of a sufficient part of the organ (not less than 25% of the volume), enough to perform the immune function. According to the current literature, most authors advocate the performance of splenectomy in patients with aneurysms located in the hilum of the spleen [16].

In our patient, the aneurysm was in the hilum of the spleen, so we performed laparoscopic splenectomy.

Laparoscopic splenectomy in the treatment of a SAA located in the splenic hilum or right next to the hilum of the spleen is a safe and effective method of treatment of this disease. Considering all the advantages and benefits of minimally invasive surgery, it should be given preference as to a method of choice, compared to open splenectomy.

Conflict of interest: None declared.

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Лапароскопска спленектомија у лечењу анеуризме артерије лијеналис – приказ болесника и преглед литературе

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САЖЕТАК

Увод Анеуризма лијеналне артерије је најчешћа висцерална анеуризма са преваленцом 0,02–10%. Такође је на трећем месту по учесталости од свих абдоминалних анеуризми. Може бити права и лажна. Јавља се чешће код жена него код мушкараца. У овом тексту приказаћемо наше искуство са болесницом старом 34 године којој је учињена лапароскопска спленектомија због анеуризме спленичне артерије локализоване у хилусу слезине.

Приказ случаја Код болеснице старе 34 године на рутинском ултразвучном прегледу абдомена дијагностиковано је проширење лијеналне артерије. Компјутеризованом томографском ангиографијом потврђено је постојање сакуларне анеуризме лијеналне артерије у хилусу слезине, димензија 24 × 17 *mm*. С обзиром на опште стање и узраст болеснице, одлучили смо се за лапароскопску спленектомију. Операција је протекла без компликација, као и постоперативни ток. Болесница је отпуштена са клинике трећег постоперативног дана.

Закључак Лапароскопска спленектомија представља сигуран и ефикасан начин лечења анеуризме спленичне артерије локализоване у хилусу слезине. Имајући у виду предности минимално инвазивног хируршког приступа, лапароскопска спленектомија се може сматрати процедуром избора у односу на класичан, отворени приступ хируршког лечења анеуризме спленичне артерије локализоване у хилусу слезине.

Кључне речи: слезина; анеуризма; лијенална артерија; лапароскопска спленектомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Metastatic atypical lung carcinoid treated with combined therapies

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SUMMARY

Introduction Lung carcinoids are considered a rare and uncommon group of lung tumors, making about 1% of all primary lung tumors. Atypical carcinoids are more aggressive than typical ones, with higher metastatic potential and worse prognosis and a 10-year survival rate of less than 60%.

Case outline In 2012, a 61-year-old male underwent the right lower lobectomy and the histopathological finding was an atypical lung carcinoid tumor. At the beginning of 2016, radiological and bronchoscopic progression of the disease was reported. Magnetic resonance imaging revealed enhanced nodular lesions compatible with liver metastases. The patient received endoluminal brachytherapy. Subsequently, the first line chemotherapy according to the cisplatin/etoposide (PE) protocol was applied. In August 2016, the somatostatin receptor scintigraphy (SRS) revealed secondary deposits with somatostatin receptor (SR) expression in the liver and lungs. The treatment with lanreotide injections was initiated. After five treatment courses, progression of the disease in the bronchial tree was verified and electro-cauterization and argon plasma cauterization of the tumor in the right main bronchus were performed. In September 2017, progression of the disease was verified again. The Oncology Board introduced the third line therapy with everolimus.

Conclusion The evidence supporting optimal treatment strategies for an atypical lung carcinoid tumor is lacking, but some recent publications indicate that multimodal treatment is associated with prolonged survival.

Keywords: lung; atypical carcinoid; somatostatin receptor; brachytherapy; everolimus

INTRODUCTION

Lung neuroendocrine tumors are classified into four categories, depending on their increasing biological aggressiveness: 1) typical carcinoid (TC), 2) atypical carcinoid (AC), 3) large-cell neuroendocrine cancer (LCNEC), and 4) small-cell lung cancer (SCLC). The guidelines for the Ki67 proliferation rate are given in the new WHO classification as the Ki67 index which amounts to 50–100% for SCLC, from 40–80% for LCNEC, 5–20% for AC, and falls below 5% for TC [1]. Lung carcinoids (LC) are rare pulmonary tumors making 1–5 % of all malignant lung tumors, having the incidence of 5–10/1,000,000 [2]. The standard treatment approach for lung carcinoid is a surgery, due to the fact these tumors are poorly sensitive to irradiation or chemotherapy [3].

TCs make up 70–90%, and ACs 10–30% of all LCs. The overall survival of patients undergoing total resection amounts to 92–100% for TCs, and 61–88% for totally resected AC. Inoperable LCs represent a considerable treatment challenge due to their poor chemo- and radiosensitivity. In addition, these tumors may reoccur or metastasize a decade after the primary resection [4, 5].

CASE REPORT

The approvals of the Committee on Ethics and of the Oncology Board were received for the purposes of this report.

A 54-year-old male with the symptoms of cough, fever, and dyspnea was admitted to the Institute for Pulmonary Diseases of Vojvodina in November 2012. The standard chest X-ray was presented with an oval opacity in the lower pole ow the right hilus (Figure 1). The chest computed tomography (CT) finding disclosed a tumorous lesion with the longest diameter of 6 cm, which infiltrated the S6 bronchus.

The endoscopy finding revealed a necrotic tumor emerging from the right Nelson bronchus, almost totally obstructing the basal bronchi. The histologic finding of the tumor biopsy sample correlated with lung adenocarcinoma. On November 12, 2012, the patient was submitted to lobectomy of the right lower lung lobe. The definite histopathology finding was tumor *carcinoides typus atypicus* (Figure 2). The TNM classification established the definite T2aN0M0 stage of the disease. Adjuvant chemotherapy was recommended, but the patient was not motivated for any additional treatment at that time. Regular postoperative Oncology Board controls were performed. All required analyses persisted normal for three years.

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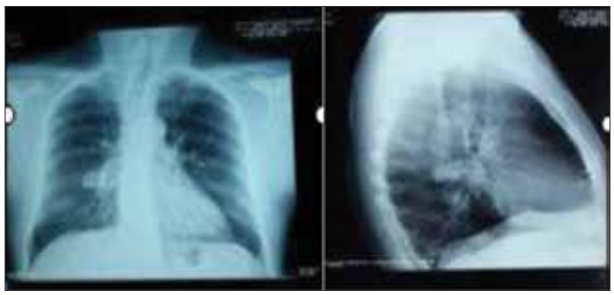

Figure 1. Chest X-ray finding: an oval opacity at the right hilus lower pole

inflammatory-type lesions at S2 on the right, accompanied with an intraluminal lesion of the intermediary bronchus. Unclearly demarcated liver lesions were also detected. The MRI finding from February 2016 was presented with multiple liver lesions characterized as secondary deposits. The whole-body positron emission tomography (PET) showed active nodes in the liver and an active lesion in the right lung (Figure 3).

Bronchoscopy performed in February 2016 revealed the following findings: tiny tumorous formations, smooth in the distal part of the trachea, and one larger smooth

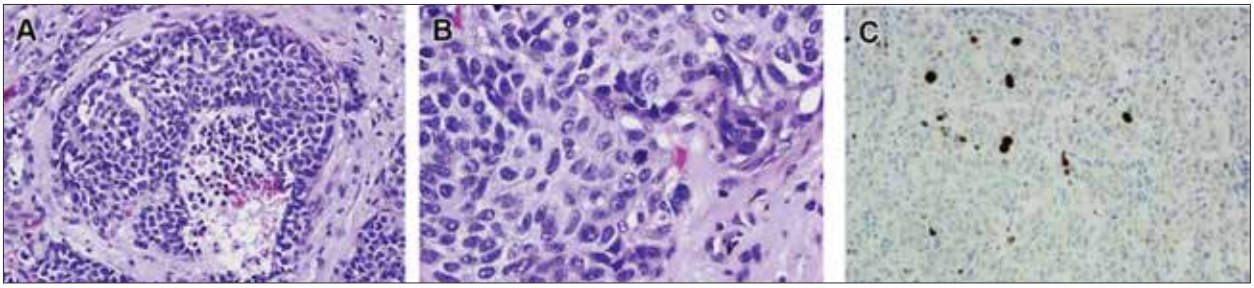

Figure 2. Atypical carcinoid: (A) punctate focus necrosis of carcinoid tumor cells and eosin; (B) a single mitosis in one tumor cell and cells with granular nuclear chromatin; (C) Ki-67 shows an intermediate proliferation rate

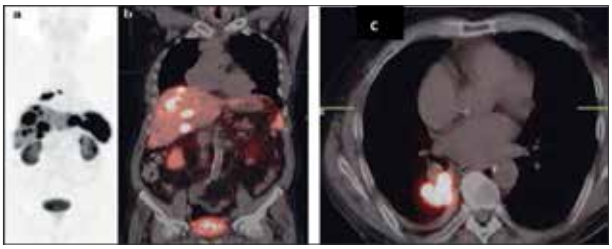

Figure 3. Magnetic resonance image of the abdomen: metastatic liver lesions, confirmed by positron emission tomography scan, which showed accumulation of radioactive fluorodeoxyglucose in the liver, as well as in the right lung

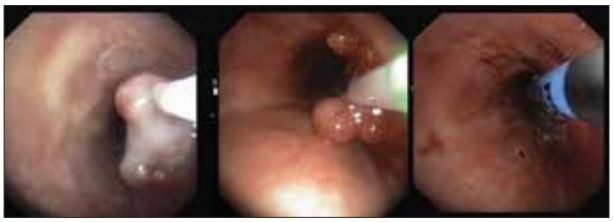

Figure 4. Endoscopic finding – the tumor removed applying the electrocautery loop; infiltrations in the tracheobronchial tree removed by argon plasm cauterization

On the regular control in November 2015, the patient had no symptoms, chest X-ray was identical to the former one, but CT of the abdomen revealed the liver involved by a few hypodense focal lesions in both lobes, probably hemangiomas. Erythrocyte pool liver scintigraphy was performed in December 2015 and detected no hemangioma-characteristic zones. The patient was scheduled for magnetic resonance imaging (MRI) of the abdomen. In January 2016, he developed fever and cough. The chest X-ray showed tiny inhomogeneous lesions in the right upper lung field and CT of the thorax disclosed infiltrative, tumor in the orifice of the upper bronchus on the right, entirely obstructing the orifices. The Oncology Board recommended endoluminal irradiation treatment, to be succeeded with chemotherapy (cisplatin/etoposide protocol). The patient completed this treatment in July 2016. On the control examination in August 2016, the finding on the lungs was in partial regression, but the finding on the liver persisted unchanged. All available histopathological samples were reassessed and they were AC of the lung, with low proliferative Ki index. The somatostatic receptor scintigraphy (SRS) was performed showing secondary deposits with SRS expression in the liver and lungs. The patient was started on lanreotide injections. After five therapy courses, progression of the disease in the tracheobronchial tree was verified. The tumor was removed by the electrocautery loop. Three months later, the tumor reoccurred at the same site so we reapplied the electrocautery loop, and then removed infiltrations in other localizations of the tracheobronchial tree by argon plasma cauterization (Figure 4). On the occasion of a new relapse episode in the right main bronchus when the lumen of the bronchus was reduced to 20%, spirometry and blood gas exchange findings persisted to be normal, but due to the latest endoscopy finding, interventional palliative bronchoscopy procedures (electrocautery of the tumor and argon plasma coagulation) were indicated.

In December 2017, the disease progression was registered in terms of an increased number and size of liver metastases, so the patient was selected for the third line treatment for AC with the everolimus drug. The patient was receiving this therapy from February to July 2018 when he developed undesirable side effects in terms of gastrointestinal symptoms and disease developed further liver progression resulting in the liver failure, so the drug

was discontinued in September 2018. At present, the patient has been receiving the symptomatic treatment with maximal supportive palliative oncological therapy.

DISCUSSION

LCs are included in the spectrum of neuroendocrine lung tumors, with a low frequency rate, ranging 0.2–2 per 100,000 inhabitants per year in the USA and Europe [6]. LCs belong to neuroendocrine lung tumors staged from the low-grade TC and intermediate-grade AC, to the high-grade LCNEC, and SCLC. TCs have less than 2 mitoses / 2 mm^2, and no necrosis, while ACs have 2–10 mitoses / 2 mm^2, and punctiform necrosis foci [7]. The diagnosis of LC is sometimes difficult to establish without immunohistochemical analyses (IHA) resulting in misdiagnosis, as it was the case in our patient, in whom the histopathological analysis of the tumor biopsy suggested lung adenocarcinoma and the definite diagnosis of carcinoid was at last established by IHA of surgically obtained biopsy samples and defined as atypical lung carcinoid. The reassessment procedure in our patient included the Ki67 proliferative index introduced in the clinical practice in 2015 by the new World Health Organization classification for neuroendocrine tumors, ranging 5–20% for ACs and amounting to < 5% for TCs. The Ki67 proliferative index in our patient's sample amounted to 15%, which additionally confirmed the IHA findings of AC [8].

Respiratory symptoms develop in centrally localized tumors, while peripheral LCs are diagnosed incidentally on the chest X-ray. Our reported patient had respiratory infection signs and dyspnea caused by centrally located tumor. The carcinoid syndrome develops in 2–5% of LCs, usually in the metastatic tumor type. The Cushing syndrome is registered in 1–6% of the affected patients [9]. The patient in our study had none of either syndrome characteristics related to hormonal hyperreactivity.

The gold standard for radiological LC detection is the contrast CT. Carcinoids usually appear as round or oval lesions with unclear or lobular margins; around 10% of the patients may develop multiple, bilobar lesions; in that case, they are always associated with calcifications [10]. The diagnostic algorithm required bronchoscopy. To obtain the mediastinal lymph node, transbronchial biopsy sample is required, which enables a precise disease staging. Real-time endobronchial ultrasound bronchoscopy has been recommended over the last decade. The latest invasive diagnostic methods also include fluorescent bronchoscopy, which precisely determines the respectability border [11, 12].

PET is strongly indicated when a local or metastatic spread of the disease, particularly AC, is suspected [13]. Our reported patient, in whom ultrasound and CT screening of the abdomen failed to establish the etiology of new liver lesions three years after the surgery, the erythrocyte pool liver scintigraphy was performed first. The MRI finding of February 2016 revealed the presence of multiple liver deposits characterized as secondary deposits. The patient was submitted to whole-body PET, disclosing active nodes in the liver and an active lesion in the right lung.

About 80% of LCs express the somatostatin-type receptor-2 and -5 (SSTR-2 and SSTR-5). In our patient, after brachytherapy as an endoscopy procedure which ablated the relapsed tumor in the right main bronchus, first line chemotherapy was applied and the patient got a few months of disease stability. When relapse of the disease occurred in the tracheobronchial tree and the liver lesions also progressed in number and size, having obtained the positive octreoscan finding, the patient was started on lanreotide injections.

TC has an excellent prognosis with the 10-year survival of over 90%, while AC is more aggressive, having a higher metastatic potential, worse prognosis, and the 10-year survival less than 60% [14, 15]. Surgical resection is the treatment of choice for patients with LCs. Advanced AC is more aggressive than TC and requires a multidisciplinary meeting review for all medical treatment decisions. The surgical treatment is not indicated in case of advanced or metastatic LCs.

European Society of Medical Oncology guidelines are similar to those of the National Comprehensive Cancer Network and recommends systemic therapy for advanced or metastatic LCs; no preferred regimen; options include cisplatin/etoposide, temozolomide with or without capecitabine, sunitinib, or everolimus; consider octreotide for symptoms of malignant carcinoid syndrome [16, 17]. The treatment with somatostatin analogues is the most frequent second-line systemic approach for patients with advanced or metastatic LCs.

Laser bronchoscopy and other invasive endoluminal procedures such as cryotherapy, argon plasma cauterization, electrocauterization, should be considered for inoperable patients or performed as a preoperative unclogging procedure. Everolimus is an mTOR kinase inhibitor which is indicated for progressive, well-differentiated, non-functional neuroendocrine tumors of lung origin that are locally advanced or metastatic [18, 19].

In the long-term course of the disease, our patient has been treated according to all the above-mentioned European and world treatment guidelines. After the applied palliative interventional bronchoscopy procedures, the patient's survival was prolonged probably due to reduced recurrent post-obstructive pneumonia. Prolonged survival was probably achieved by the use of other therapeutic modalities such as cisplatin/etoposide chemotherapy, somatostatin analogues, and, lastly, the use of everolimus.

Conflict of interest: None declared.

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Метастатски атипични плућни карциноид третиран комбинованим терапијама

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САЖЕТАК

Увод Плућни карциноиди чине ретку групу плућних тумора са заступљеношћу око 1% свих примарних плућних тумора. Атипични карциноид плућа је агресивнији у односу на типични, са већом могућношћу метастазирања и лошијом прогнозом, а 10-годишње преживљавање је мање од 60%.

Приказ болесника Године 2012. код 61-годишњег болесника урађена је десна доња лобектомија, а дефинитивни патохистолошки налаз је одговарао атипичном плућном карциноиду у почетном стадијуму болести. Почетком 2016. године радиолошки и бронхоскопски је потврђен рецидив болести у бронху. Магнетна резонанца абдомена потврдила је присуство нодуларних лезија које су одговарале јетреним метастазама. Болесник је тада примио брахитерапију захваћеног дела бронхијалног стабла и хемиотерапију по протоколу цисплатин/етопозид. У августу 2016. године сцинтиграфија соматостатинским рецепторима је показала експресију ових рецептора у плућима и јетри и болесник је отпочео терапију са хемиотерапеутиком ланреотиде. После пет циклуса ове терапије јавила се нова прогресија болести у бронхијалном стаблу, те се урадила електрокаутеризација и аргон-плазма каутеризација тумора. Нова прогресија болести настала је у септембру 2017. године, када је болесник започео терапију са леком еверолимус.

Закључак Оптимални терапијски водичи за лечење атипичног плућног карциноида нису утврђени, а нови објављени радови указују на неопходност његовог мултимодалитетног лечења, чиме се омогућава дуже преживљавање ових болесника.

Кључне речи: плућа; атипични карциноид; соматостатински рецептори; брахитерапија; еверолимус

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

An unperformed autopsy does not exclude the possibility of proving a physician's error

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SUMMARY
Introduction/Objective Clarifying cases involving suspicious natural death and all forensic problems connected to such cases is possible only with the aid of a timely and adequately performed autopsy. The objective of this paper, however, is to point out the fact that it is possible to prove the existence of a physician's error, even when an autopsy had not been performed.
Case report The Emergency Medical Service (EMS) team had been dispatched to respond to a call for help by a 53-year-old woman, complaining of chest pain, shortness of breath, and dizziness. The pain was located in the center of her chest and would increase in response to palpation, change of body position, and deep breathing. The physical examination was normal. The EMS physician concluded that it was not necessary to perform electrocardiography (ECG). Forty minutes later, the EMS team was dispatched to see the same patient again, this time for suspected cardiac arrest. Protocol-based cardiopulmonary resuscitation (CPR) for asystole was performed, without success. After 30 minutes, CPR was discontinued and the patient was declared deceased. Although the patient's relatives did not allow an autopsy to be performed, they did send a claim to the Health Inspector at the Ministry of Health of Serbia, demanding an internal review of the physician's professional work. It was concluded that the physician should have performed ECG, but that the true cause of death could only have been determined through a timely and adequately performed autopsy.
Conclusion An unperformed autopsy does not exclude the possibility of proving a physician's error.
Keywords: absence; evidence; autopsy; physician's error

INTRODUCTION

A physician's error is defined by the Law on Health Care of the Republic of Serbia, Article 186, Paragraph 1 [1]. According to the Law, a professional error implies unconscientious treatment, neglecting of professional duties in providing healthcare, failure to comply with defined rules and professional skills in providing healthcare which leads to injury, damage, deterioration of health or loss of body parts in a patient. [1]. This legal norm represents the basis for assessment of possible ethical and legal responsibilities of doctors whose errors had caused a worsening in patients' health or lethal outcomes. In Germany, the Robert Koch Institute proclaimed that 40,000 complaints on suspected physician's errors are made yearly and that of those more than 12,000 remain unconfirmed. They also concluded that more people are affected by physician's errors than by traffic accidents each year [2, 3]. Researchers in the USA concluded in the year 2000 that 44,000–98,000 patients die annually as a result of physicians' errors [4, 5].

The only way to truly discover the manner and cause of sudden death is through autopsy findings [6]. In cases where a person had asked for medical help, which was then followed by a lethal outcome, a question is sometimes put forward whether the physician had done everything, diagnostically and therapeutically, that was within his power and in accordance with the principles of modern medical science and practice [7, 8]. Accordingly, it is possible to initiate a criminal justice procedure for the criminal offence of medical malpractice (Article 251 of the Criminal Code of the Republic of Serbia). Clarifying cases involving suspicious natural death and solving forensic problems connected with such cases is possible only with the aid of a timely and adequately performed autopsy [9]. However, if an autopsy had not been performed, that does not necessarily testify that a medical error did not exist [10]. In such cases, the judicial decision is mostly based on forensic evaluation, and the duration of the judicial process and correctness of the verdict mostly depend on the quality of performed medical expertise [11].

The objective of the paper is to point out that even if an autopsy had not been performed, there are still possibilities to prove that a physician's error did occur.

CASE REPORT

The Emergency Medical Service (EMS) team was dispatched at 2:40 p.m. to respond to a call for help by a 53-year-old woman, suffering from asthma, chronic gastritis, and cholecystitis. On team arrival, the patient was conscious, alert and oriented. She complained of chest pain, shortness of breath, and dizziness. Her pain was located in the center of her chest

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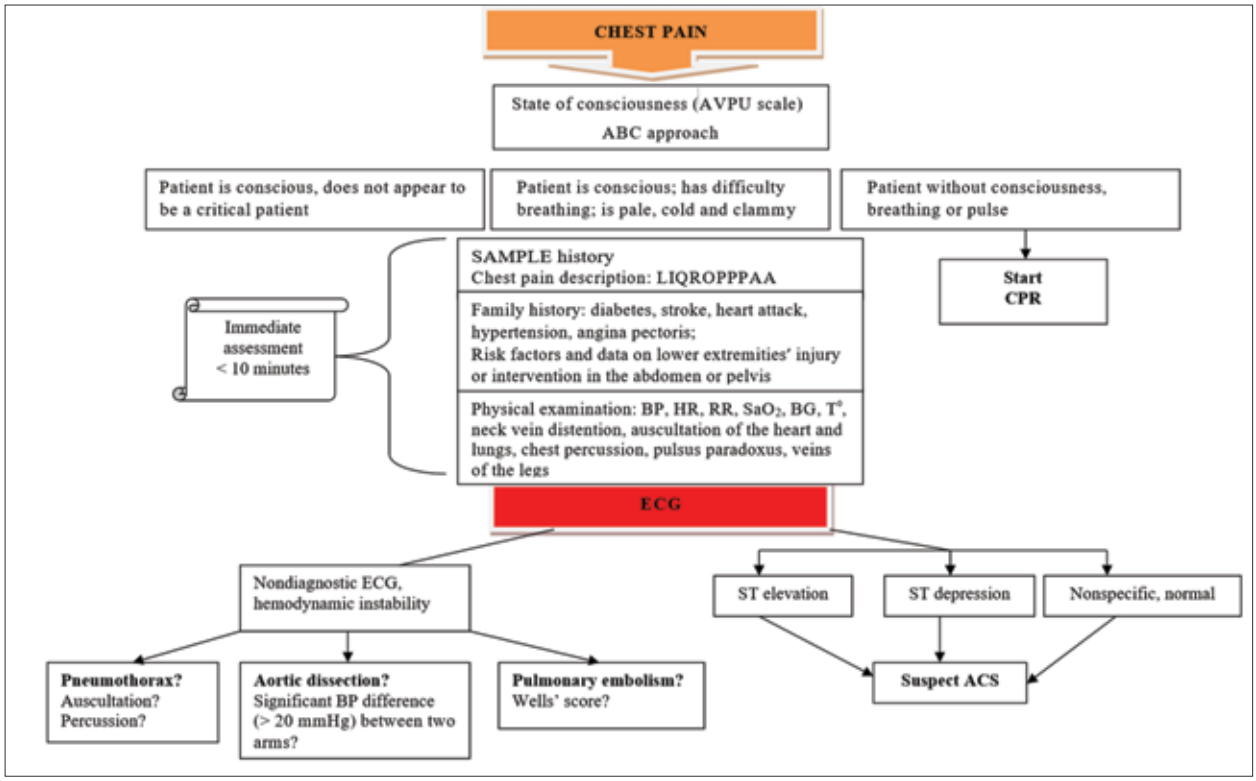

Figure 1. Prehospital assessment of chest pain; AVPU (A – alert; V – verbal response; P – response to pain; U – unresponsive); ABC (A – airway, B – breathing, C – circulation); SAMPLE [S – signs/symptoms, A – allergies, M – medications, P – past illnesses, L – last oral intake (last menstrual cycle), E – events leading up to present illness]; LIQROPPPAA [L – location, I – intensity (on the scale 0–10), Q – quality, R – region and radiation, O – onset, P – precipitation events, P – progression, P – previous episodes, A – alleviating factors, A – aggravating factors]; BP – blood pressure; HR – heart rhythm; RR – respiratory rate; SaO_2 – oxygen saturation

and would increase in response to palpation, change of body position and deep breathing. The patient was of unchanged skin color, afebrile, eupneic, normofrequent (pulse 88 beats/minute) and normotensive (BP 130/80 mmHg). On auscultation, her heart was of regular rate and rhythm, with normal S1 and S2, without murmurs, rubs, or gallops. Her breath sounds were diffusely decreased bilaterally with prolonged expirium, without crackles, rhonchi, or wheezes. Her abdomen was soft, non-tender and non-distended, with normoactive bowel sounds and without hepatosplenomegaly. Her extremities were symmetric in appearance with preserved motor and sensory function, without deformities or edema. Neurological findings were within normal limits. Based on many years of experience, the physician evaluated that it was not necessary to perform an electrocardiogram (ECG). The patient was treated with intramuscular injections of diclofenac and dexasone and advised to call the EMS again should her condition deteriorate. They left at 3:10 p.m. At 3:46 p.m. the EMS team was dispatched to the same address again, this time to deal with a suspected cardiac arrest. The patient was now unconscious, not breathing, and had no pulse. The defibrillator monitor presented asystole. Cardiopulmonary resuscitation (CPR) was performed adhering to non-shockable rhythm protocol, but it was unsuccessful. At 4:05 pm, CPR was discontinued and the patient was declared deceased.

At the time, the patient's family did not allow an autopsy, but later they did send a claim to the Healthcare Inspector at the Ministry of Health of the Republic of Serbia demanding an internal review of the physician's professional work. Complete documentation was analyzed: the order for review issued by the Healthcare Inspector, the complaint of the patient's family, transcripts of the recorded conversation with the EMS 194 Dispatch Center, the physician's reports, the physician's statement and the statement given by the Head EMS Technician in charge of equipment, who testified that the EMS team were in possession of all the necessary equipment to perform an ECG and that the equipment was in working order. It was concluded that the physician should have performed an ECG, but that the true cause of death remains unknown since the autopsy had not been performed.

DISCUSSION

Physician's errors are defined by the Criminal Code (CC) of the Republic of Serbia under the heading of Medical Malpractice (CC, Article 251) [12]. According to this CC article, "a doctor who in providing medical services uses an evidently inadequate means or an evidently unsuitable treatment or fails to observe appropriate hygiene standards or evidently proceeds unconscientiously and thereby causes deterioration of a person's health, shall be punished by imprisonment of three months to three years." "Evidently inadequate means or evidently unsuit-

able treatment" covers anything that the physician might have done or not done that is drastically contrary to the generally accepted contemporary principles of medical science and practice, in other words all that represents a cardinal mistake which falls outside the frame of medical tolerance [13]. As chest pain can signify an urgent medical condition, according to the contemporary guidelines for healthcare of patients with chest pain, a 12-channel ECG (Figure 1) is the most significant method for reaching the true diagnosis and applying adequate therapy [14].

The charge of medical malpractice can also be put forward in cases when an undiagnosed disease results in lethal outcome at a hospital, particularly when it is estimated that the death could have been prevented by using timely and adequate therapeutic measures. Inability to reach a correct diagnosis can sometimes be the consequence of non-specific clinical features of the disease or it can be the result of the physician's failure to correctly interpret the patient's complaints [15]. However, it is most commonly the case of failing to apply all the necessary diagnostic procedures available (ECG in this case) in order to make a specific diagnosis. Pejaković [9] states that superficiality and incompleteness are elements of medical negligence. On the other hand, even when the physician had obviously acted with negligence, a deterioration in the patient's health could occur for completely different reasons, for example an unrelated undiagnosed disease, etc. It can also happen that the deterioration of the patient's health occurred only partially due to the physician's error and partially as a result of some other causes that were not influenced by the physician's incorrect diagnosis or treatment. It is very difficult to determine to what extent the deterioration in the patient's health was influenced by the physician's error and to what extent by other factors [10]. All these and other circumstances must undergo evaluation and if criminal responsibility is to be sought, undeniable proof must be found.

According to an earlier analysis of legal records, it was discovered that out of 147 cases in which patients had died, which underwent analysis for suspected medical malpractice, autopsy was performed in only 36% [10]. Bove and Iery [16] found that information gained as a result of an autopsy can be helpful to either the plaintiff or the defendant or can even be neutral in a given case. Especially noteworthy is the finding that in 61% of all the cases in which the reviewers concluded that the information provided by the autopsy favored the plaintiff, the defendant was none the less acquitted of the charge of medical malpractice. Conversely, in 100% of all the cases in which reviewers thought that the autopsy findings favored the defense, the defendants were acquitted.

The definitive judgement on the presence or absence of the criminal act of medical malpractice is made by the court. Occasionally, due to insufficient evidence or being subject to the statute of limitations, the public prosecutor can abandon criminal pursuit of the physician [11].

In the presented case, an error was made to accept the request of the relatives and no autopsy had been performed. It should be emphasized that the will of the members of the deceased's family has no bearing on the decision to perform an autopsy, regardless of whether it is a medico-legal or clinical autopsy. Unfortunately, our current medical practice has often acted contrary to the regulations, because doctors unjustifiably decided that, on the basis of a personally signed request by one of the family members, a clinical autopsy would not be performed. Namely, there was an erroneous presumption that family members cannot forbid a medico-legal autopsy, but do have the right to ban a clinical autopsy, which is not at all true. In other words, according to current legal provisions, the family of the deceased has no legal right to suspend an autopsy if it is indicated by medical and/or legal criteria.

According to legal regulations in Serbia, each deceased is to be examined by a medical doctor, who is to determine the time and cause of death (Law on Health Care of the Republic of Serbia, Article 203, Paragraphs 1 and 2) [1]. The said medical doctor is obligated to immediately contact the police should he not be able to determine the cause of death based on available medical records, as described by the Law on Health Care of the Republic of Serbia, Article 204, Paragraph 1. [1]. The police would then notify the public prosecutor, whose obligation it is to determine the need for an autopsy. According to the Law on Health Care of the Republic of Serbia, Article 206, Paragraph 2, an autopsy should be obligatorily performed at the request of a member of the immediate family of the deceased person or if a death occurs in the course of a diagnostic or therapeutic procedure or even after the procedure if there is reason to believe that the death occurred in connection with the said procedure [1].

The family refused to allow an autopsy to be performed, but later put in a request for a review of the physician's performance to the Health Inspector at the Ministry of Health. Since only a timely and adequately performed autopsy can determine the cause of death, as well as the elements of alleged medical malpractice as described in Article 251 of the CC of Serbia, the fact that the autopsy had not been performed ruled out the possibility of proving a cause and effect relationship between the actions of the physician and the deterioration of health of the deceased [17]. Therefore, the physician in question could not be charged with a crime.

However, through analysis of all available documentation, it was possible to determine that the physician had not complied with generally accepted contemporary guidelines of medical science and practice in diagnosis and treatment of chest pain, which state that a 12-channel ECG (Figure 1) is the most significant method for making the right diagnosis and applying adequate treatment when dealing with chest pain.

Therefore, on the basis of medical records and other collected evidence, it was possible to conclude that this was a case of a physician's error, even though the autopsy had not been performed and it was not indisputably proven that this error had anything to do with the patient's deterioration of health and ultimately death.

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Неизвршена обдукција не искључује могућност доказивања лекарске грешке

Слађана Анђелић

Градски завод за хитну медицинску помоћ, Београд, Србија

САЖЕТАК

Увод/Циљ Разјашњење случајева нејасне природне смрти и решавање свих судскомедицинских проблема у вези са тим случајевима могући су само на основу благовремено и адекватно извршене обдукције.

Циљ рада је да укаже на чињеницу да неизвршена обдукција не искључује могућност доказивања лекарске грешке.

Приказ болесника Екипа хитне медицинске помоћи упућена је на интервенцију код болеснице старе 53 године због бола у грудима, отежаног дисања и несвестице. Бол је био локализован у средњем делу грудне кости, појачавао се на додир, при промени положаја тела и са дубоким дисањем. Физикални налаз по системима је био уредан. Лекар хитне медицинске помоћи је проценио да не треба урадити ЕКГ. После 40 минута екипа је поново била упућена код ове болеснице због сумње да је дошло до срчаног застоја. Кардиопулмонална реанимација спроведена по протоколу за асистолију била је безуспешна, те је после 30 минута проглашен смртни исход. Иако породица није дозволила обдукцију, поднела је жалбу Министарству здравља Србије, које је одредило унутрашњу проверу квалитета стручног рада доктора. Закључено је да је требало да лекар уради ЕКГ, али да је једино обдукцијом могао бити утврђен прави узрок смрти.

Закључак Неизвршена аутопсија свакако није доказ непостојања лекарске грешке.

Кључне речи: одсуство; доказ; обдукција; лекарска грешка

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Gout – asymptomatic hyperuricemia with/without asymptomatic monosodium urate crystal deposition: to be treated or not?

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SUMMARY
Elevation of serum uric acid level without clinically visible arthritis (known as asymptomatic hyperuricemia) is not traditionally considered to be gout disease, but only a possible cause of it, even though it may be accompanied by tissue uric acid crystal deposition. On the other hand, gout is traditionally recognized as recurrent, overt arthritis, visible only after a long period of time due to uric acid accumulation in joints. Advanced imaging techniques have substantially changed the perception of this problem, identifying gout as a low-grade chronic inflammatory disease from the very beginning, visible only by phases of acute arthritis attacks. According to ultrasonography, uric acid crystal hyperechoic aggregates (tophi) are seen not only in the symptomatic gout disease phase, but also in the preceding – asymptomatic (latent) – gout phase. New perception of the problem was approved by the recently described NETs (neutrophil extracellular traps) phenomenon. Also, hyperuricemia has recently been identified as a systemic disorder, responsible not only for the apparent gout arthritis, but also for the renal and cardiovascular disease occurrence and progression.
Positive effect of urate-lowering therapy (xanthine oxidase inhibitors and uricosurics) on hypertension and chronic kidney disease indicates a possibility of its utility in asymptomatic hyperuricemia and asymptomatic gout therapy, apart from the use in clinically manifested gout treatment and for certain conditions, such as tumor lysis syndrome.
Keywords: asymptomatic hyperuricemia; monosodium urate crystal deposition; gout; advanced imaging studies; ultrasonography; NETosis

INTRODUCTION

Gout is an inflammatory rheumatic disease, characterized by monosodium urate (MSU) crystal deposition in joints and connective tissues generally, localized periarticularly or subcutaneously. According to the current diagnostic algorithm (New York, Rome, and the American College of Rheumatology criteria), the acute gout arthritis attack is traditionally required for gout diagnosis [1]. The classical gout disease goes through four linear but discontinuous phases: 1. asymptomatic hyperuricemia phase (AHU); 2. recurrent acute arthritis phase; 3. intercritical phase (between two episodes of gout arthritis); and 4. chronic tophaceous gout phase [2]. Actual therapeutic paradigm does not treat the first phase (AHU) patients, and therapy candidates are only those with a visible gout arthritis attack (at least one) or patients with chronic, visible, tophaceous gout [3–6].

Recently discovered phenomenon of NETosis (NET – neutrophil extracellular traps) in gout has enabled a closer evaluation of AHU [7–10], together with advanced imaging techniques [11–15], *in vivo* and *in vitro* laboratory studies, retro and prospective cohort studies and randomized interventional trials. They have all shown that UA is not only a marker, but also a mediator of hypertension and renal dysfunction, thus offering arguments for the present diagnostic and therapeutic recommendations amendment proposal.

PATHOGENESIS OF GOUT NETOSIS

Hyperuricemia (HU) is the key risk factor for gout development. However, only 22% of people with extremely high level of serum uric acid (SUA) (more than 535 μmol/L, i.e. 8.9 mg/dL) will develop symptomatic gout in a period of five-year follow-up [4]. In contrast, 5% of gouty patients will never be presented with the SUA elevation.

Additional risk factor for gout development is monosodium urate crystal deposition in tissues, followed by the local tissue reaction presented in persons with high SUA level and even in those with normouricaemia. Crystal deposition depends on UA solubility, which is variable and decreased by high serum uric acid concentration, as well as by high acidity and low temperature.

Initiated by UA serum supersaturation, joint and connective tissue localized crystallization leads to the activation of cytokines and other inflammatory mediators. Interleukin 1β is the

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Table 1. Echosonographic evidence in asymptomatic hyperuricemia

Publication	SUA in AHU mg/dL (μmol/L)	US evidence	Joints	Frequency (%)	Controls	Gout
Puig et al.[1]	8.5 (505.6)	Tophi	Knees and TC	12/35 (34%)	/	/
Howard et al.[2]	8 (475.8)	Double contour and/ or tophi	Femoral cartilage and MTP1	5/17 (29%)	1/19 (5%)	7/14 (50%)
Pineda et al.[3]	8.1 (481.8)	Double contour	Knees and MTP1	17/100 (17%) knees; 25/100 (25%) MTP1	0/104 knees 0/104 MTP1	/
De Miquel et al.[4]	8.5 (505.6)	Double contour or hyperechoic spots	Knees and feet	11/26 (42%); 9/11 (81.8%) + crystal	/	/

SUA – serum uric acid; AHU – asymptomatic hyperuricemia; US – ultrasound; TC – talocrural joint; MTP1 – first metatarsophalangeal joint:
[1]Nucleosides Nucleotides Nucleic Acids. 2008; 27:592–5;
[2]Arthritis Care Res. 2011; 63:1456–62;
[3]Arthritis Res Ther. 2011; 13(1):R4;
[4]Ann Rheum Dis. 2012; 71:157–8

principal and the most important proinflammatory cytokine, produced by residential cells of connective tissue, after the NALP3 inflammasome activation. Macrophage phagocytosis of monosodium urate crystals leads to intracellular hypernatremia, hypervolemia and consequently hypokalemia, as well as to cascade of NALP3 inflammasome activation and caspase-dependent activation of IL1β path (from proIL1β). Secretion of IL1β attracts neutrophils, which support the inflammatory response by proinflammatory mediators' production and excretion [7].

As it is a well-known fact that signs and symptoms of inflammation do not follow TOPHUS (pathognomonic structure in gout patients) presence in patients with chronic gout, the following question was raised: Which is the way that leads to the resolution of the inflammatory response and pacification of crystals in TOPHUS? This question has been waiting for an answer for a long time.

According to recent *in vivo* and *in vitro* studies, the induction and resolution of gout inflammation is orchestrated by both monocytes and granulocytes. Neutrophils act not only through phagocytosis, intraphagosomal digestion and secretion of inflammatory mediators, but also through creation of the neutrophil extracellular traps (NETs), which are defined as the active cell death, different from apoptosis and necrosis [7–10]. NET histochemically represents extracellular DNA of neutrophils and proteolytic enzymes (elastase, cathepsin G, myeloperoxidase-MPO complex). NET structure limits the spread of the aggressive entity (in this case, crystal) both chemically and mechanically by inflammatory mediators' proteolytic degradation. MSU crystals are the most powerful drivers of NETosis under almost all physiological conditions, including whole blood and plasma.

Patients with acute uric arthritis resolution are presented with robust NETosis (activation and release of NETs) in the synovial fluid as well. Furthermore, TOPHUS is traditionally associated with poorly controlled chronic disease and can actually be found in all stages of the disease. TOPHUS is composed not only of natrium sodium urate crystals, but also of extracellular DNA and neutrophil proteolytic enzymes that neutralize crystals mechanically and chemically (has, in fact, all the characteristics of the above-mentioned NET aggregate). Adenosine triphosphate disodium (ATP) and lactoferrin, which are released during the NET formation process, are extremely important for the inflammatory reaction resolution. Activation of extracellular nucleotides from mononuclear cells initiates the necrotic cell clearance, while lactoferrin serves as a specific inhibitor of the polymorphonuclear migration.

ULTRASONOGRAPHY IN GOUT AND ASYMPTOMATIC HYPERURICEMIA

Owing to advanced imaging techniques (ultrasound, magnetic resonance imaging, computed tomography), asymptomatic synovial sheath inflammation can be seen in joints that have never been presented with the traditional, clinically visible gout arthritis [11–15]. It can also be presented in the so-called intercritical period (between two apparent gout arthritis attacks). Furthermore, ultrasound displays MSU crystal deposition in tissues as a structural change of the articular cartilage, showing either a double contour sign or the TOPHUS formation, and can be found not only in the inflamed joints, but also in joints that have never been affected by overt arthritis [13] (Table 1). Sensitivity of the ultrasound urate tissue deposition finding (double contour sign or TOPHUS) is variable and ranges 20–90%, which depends on previous therapy (treated or not), data availability (blinded or unblinded research), study type (prospective or retrospective), type of observed joints, etc. The specificity is 98–100%.

The most acceptable balance of sensitivity and specificity was reached by Naredo et al. [11] ultrasound examination standard recommendation, which demands evaluation of six anatomic structures bilaterally and simultaneously (12 regions): three structures for TOPHUS hyperechoic aggregates – one joint (radiocarpal) and two tendons (patellar ligament and the triceps muscle tendon) – and three cartilages for the double contour sign – first metatarsophalangeal joint, second metacarpophalangeal joint and calcaneal or femoral condylus cartilage. The sensitivity of Naredo et al. [11] examination was 85%, specificity 83%, positive predictive value 92%, and negative predictive value 71%.

The new possibilities of ultrasound examination have substantially changed the perception of gout, which seems to be a chronic inflammatory disease from the very beginning, only expressed by different levels of activity (visible or not). Acute, vigorous gout arthritis is just a tip of the iceberg which enables us to see gout (Figure 1), just like an

osteoporotic fracture makes the osteoporosis visible. Since the advanced imaging techniques have enabled diagnosis of gout in its subclinical, latent, inapparent form, the question of the asymptomatic disease therapy was raised. Here, we have offered some arguments that asymptomatic hyperuricemia with MSU crystal deposition could be regarded and treated as the gout disease.

HYPERURICAEMIA: THE PRINCIPAL RISK FACTOR FOR METABOLIC SYNDROME, HYPERTENSION, AND RENAL FAILURE OCCURENCE

UA has been identified as not only the marker, but also a mediator of hypertension, cardiovascular morbidity and progressive decline in renal function by a number of recently reported studies from animal models, clinical retro and prospective observational studies, and randomized intervention trials [16–27]. According to the latest data, the paradigm of the causative association between hyperuricemia and cardiovascular and chronic kidney disease seems to have progressed from skepticism to true evidence of relationship [17, 18]. However, therapy remains controversial [19].

UA is known as the major antioxidant agent in human plasma. However, its antioxidant nature comes to its own opposite within the cell, where it paradoxally converts to pro-oxidant agent, which mostly targets lipids [low-density lipoproteins (LDL) and membranes] [16]. Cirillo et al. [20] have noticed elevated SUA level in patients with metabolic syndrome and have concluded that uric acid is not just a link in the metabolic syndrome chain, but plays a crucial role in its development. UA-caused adipose tissue fat cell oxidation promotes insulin resistance that leads to hypertension, visceral obesity, hypertriglyceridemia, dyslipidemia and hyperglycemia.

In addition to LDL oxidation caused by the prooxidant SUA effect, the atherosclerotic process is started by the nitric oxide production (also known as the endothelium-derived relaxing factor – EDRF), which leads to endovascular inflammation and inflammatory mediators cascade reaction primarily. LDL oxidation and vasoconstriction lead to stable atherosclerotic plaque formation, which becomes unstable in time, resulting in a well-known diversity of cardiovascular events.

Furthermore, hyperuricemia-activated renin-angiotensin-aldosterone system adds to hypertension development. Kidney UA crystal deposition promotes stone occurrence, tubulointerstitial nephritis and fibrosis which additionally leads to hypertension, renal function decline and UA serum level increase. It is not exactly known which process serves as a trigger factor in the newly created *circulus vitiosus*, but certainly there is a chain that should be interrupted (Figure 2).

The impact of hypo-uricaemic therapy on the cardiovascular events' occurrence risk is not fully understood yet. An improvement of endothelial function has been shown by a small number of interventional trials using XO inhibitors. In patients with chronic heart failure and HU, vasodilatation enabled by XO inhibitors improves the blood flow. Indeed, significant blood pressure decline was observed in patients who received antihypertensive therapy combined with allopurinol, as compared to antihypertensives alone [25]. Finally, a significant reduction in cardiovascular morbidity and mortality was shown in gout patients on higher allopurinol dosage and with lower SUA level, according to a large retrospective cohort study [26].

Allopurinol-achieved low SUA level is not always correlated with an improvement of the endothelial function. Also, recent investigations advised caution when using allopurinol, since it can have side effects, such as induced gout attacks, elevated aminotransferases, and cytopenia) [19]. On the other hand, uricosuric agents such as probenecid and benzbromarone did not show similar benefit on endothelial function [28].

Management of asymptomatic hyperuricemia has been approved in Japan only, for people with SUA level more than 9 mg/dL [29]. This subject is very complex, since

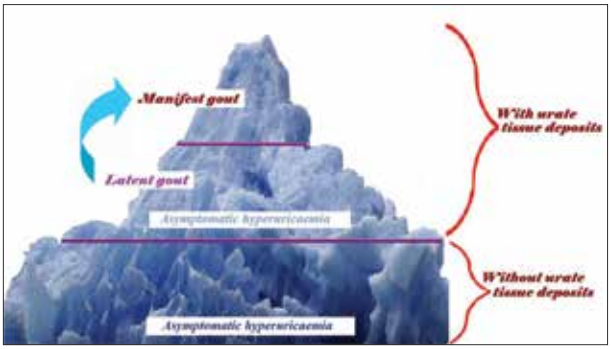

Figure 1. The course of the gout disease (iceberg);
Tip: symptomatic disease, traditionally presented with clinically visible arthritis with/without monosodium urate crystal deposition;
Middle: latent gout, presented as asymptomatic hyperuricemia and monosodium urate crystal deposition (as seen by advanced imaging techniques) that could be considered as gout as well, thus raising the question of therapy (further described in the text);
Basis: asymptomatic hyperuricemia without urate tissue depositions leads to controversies in terms of therapy, due to promotive effect of this state on cardiovascular events and decline in renal function

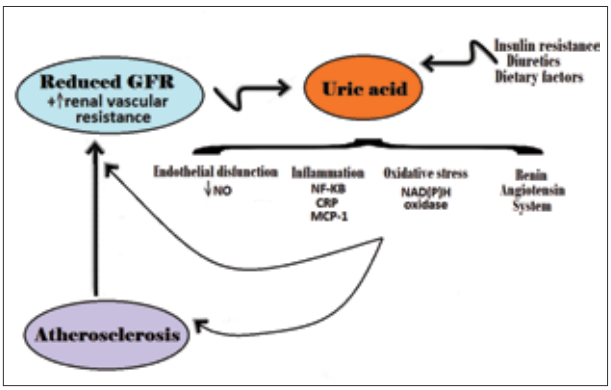

Figure 2. *Circulus vitiosus* made of high serum uric acid level, atherosclerosis, and renal function decline;
GFR – glomerular filtration rate; NO – nitric oxide; NF-KB – nuclear factor kappa-light-chain-enhancer of activated B cells; CRP – C-reactive protein; MCP-1 – monocyte chemotactic protein-1; NAD(P)H – nicotinamide adenine dinucleotide phosphate-oxidase; source: Am J Kidney Dis. 2012, The National Kidney Foundation

there is no reliable data to make strong international recommendations yet. The most recent European League Against Rheumatism evidence-based recommendations for the management of the gout state that recent studies have yielded conflict the results regarding asymptomatic hyperuricemia treatment [3].

Indeed, genetic evidence based on conventional and novel Mendelian randomization approaches suggest a modest, if any, causal effect of SUA concentration on the development of cardiovascular disease [30]. A collaborative group from Europe, New Zealand, United States, etc. has collected more than 400,000 samples from gout patients to perform the largest genome-wide associated study ever conducted in people with gout and the data should be available by the end of 2019 (thanks to prof Richette Pascal).

CONCLUSION

Here we have offered arguments that asymptomatic hyperuricemia with tissue urate crystal deposition (latent gout) could be regarded as gout and treated accordingly, bearing in mind the promotive effects of hyperuricemia on hypertension, cardiovascular disease, and renal disease. Further studies identifying the guidelines for the therapy regime for asymptomatic hyperuricemia would be beneficial.

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Гихт – асимптоматска хиперурикемија са асимптоматским таложењем кристала мононатријум-урата и без њега: да ли лечити?

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САЖЕТАК

Повишен ниво мокраћне киселине у серуму без клиничких знакова артритиса (познат као асимптоматска хиперурикемија) традиционално се не схвата као гихт, већ само као могућа (претходна) фаза ове болести, мада може бити повезан са депозицијом кристала мокраћне киселине у ткивима. С друге стране, гихт се традиционално препознаје као рецидивирајући, видљиви артритис који се јавља само после дужег трајања болести због акумулације мокраћне киселине у зглобовима.

Напредне технике снимања суштински су промениле перцепцију овог проблема, показавши да је гихт од самог почетка хронично инфламаторно обољење ниског степена активности, а да га епизода акутног артритиса само чини видљивим. Као што је доказано ултразвуком, интраартикуларни хиперехоични агрегати кристала мокраћне киселине (тофуси) налазе се не само у симптоматској фази гихта већ и у претходној – асимптоматској (латентној) фази. Нову перцепцију проблема потврдио је недавно описан феномен *NET*-озе (неутрофилних екстрацелијских замки). Такође, хиперурикемија се у последње време сматра системском болешћу, одговорном не само за видљив напад артритиса већ и за настанак и прогресију реналних и кардиоваскуларних болести.

Позитиван утицај лекова који смањују ниво мокраћне киселине у серуму (инхибитора ксантин-оксидазе и урикозурика) на хипертензију и хроничну бубрежну болест индикује могућност њихове примене у лечењу асимптоматске хиперурикемије и асимптоматског гихта, поред редовне употребе код клинички манифестног гихта и у одређеним стањима (као што је синдром лизе тумора).

Кључне речи: асимптоматска хиперурикемија; таложење кристала мокраћне киселине; гихт; имиџинг студије; ултразвук; неутрофилне екстрацелијске замке

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Historical aspects of left-handedness

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SUMMARY
Lateralization is one of the central questions in neurology, neuropsychology, and other related scientific disciplines. There has been very little change in the proportion of left-handers since the Upper Paleolithic Age about 10,000 years ago and it is estimated to be around 10%. As the history of human thinking has developed from superstition to science, the explanation of left-handedness transformed from "devil's work" to neurological specificity. This paper presents this very interesting historical change by analyzing the data on left-handedness and the attitudes towards it in human societies from prehistory to today. Even in a relatively open-minded society, parents and teachers may encourage a left-handed child to switch to right-handedness to make their lives easier in a largely right-handed world. On the other hand, left-handedness is increasingly seen as a special gift, and left-handed people have started to favor themselves as more competent in relation to the right-handed people.
Keywords: laterality; handedness; history

INTRODUCTION

Hand dominance is a tendency to use one hand rather than the other for certain fine motor activities that require the use of only one hand [1]. Human hands are architecturally symmetrical, but they markedly differ in the tendency to perform various manual activities more frequently with one hand rather than the other. In the majority of people, the right hand is dominant [2].

Large intra-individual variation in hand preference makes a well-known dichotomous distinction on left and right-handed people insufficient for a clearer understanding of this phenomenon. Some people are inconsistent in hand preference, using a preferred hand for one and non-preferred hand for another manual activity. Such an inconsistency in hand preferences is usually described as "mixed-handedness." In contrast to this, "ambiguous handedness" represents inconsistency in the use of the dominant hand of the same manual activity. Another relevant distinction is related to the degree of hand preferences. Two right-handed people may differ in the degree of preference; one always uses the preferred hand for most activities, whereas the other sometimes uses the non-preferred hand for certain activities [3].

To date, many aspects of hand dominance such as evolution, etiology, geographic variations, presence in different social communities and in time, remain unclear. Many scientists think that lateralization is one of the central questions in neurology, neuropsychology, and other related scientific disciplines [1].

As the history of human thinking has developed from superstition to science, the explanation of left-handedness transformed from "devil's work" to neurological specificity. The objective of this paper is to present this interesting historical change by analyzing the data on the presence of left-handedness and the attitudes towards it in human societies from prehistory to today.

PREHISTORY

Numerous studies have been carried out to answer the question when, where, and how man became right-handed [4]. According to archaeologist Nicholas Toth, as long as 1.5 million years ago, more than half of the early stone tools were chiseled with the right hand [5]. Another proof of prehistoric handedness is a 1.6-million-year-old *Homo ergaster* skeleton found in Kenya by Richard Leakey and his team; it shows some evidence of right-handedness in the length of the ulna bones and the depth with which the deltoid muscles attach to the clavicle [6].

Furthermore, *Australopithecus africanus* from 2–3 million years ago used to smash the skulls of baboons the way the right-handed people do [7]. An analysis of the shapes of various hominid tools about 500,000 years old found that most of them were made and used by right-handers. The remains of an old Neanderthal, 35,000 years old, show a 93% prevalence of right-handedness [8].

Interestingly, there has been very little change in the proportion of left-handers, which continues at around 10%, since the Upper Paleolithic Age, around 10,000 years ago [9]. An analysis of handedness in some skilled actions, from throwing a spear to needlework, by Stanley Core and Clare Porac, shows a remarkably

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consistent record of around 10% left-handedness going back over 5,000 years. This is amazingly similar to the proportion of left-handed people in the human population today [10, 11].

ANCIENT HISTORY

Right-handedness was also dominant in ancient civilizations, from Greece and Rome to China, Egypt, and Mesopotamia. It was a privilege to be on someone's right-hand side. In almost all of these cultures, the right hand was used for ceremonies and for eating. The inhabitants of Mesopotamia considered left-handedness a punishment from the gods. Strongly anti-left ancient Egyptians often depicted their enemies as left-handed, while they were the righteous dextral [9].

Each of the 10 first principles of the early Greek mathematician and philosopher Pythagoras comprised pairs of opposites, and it comes as no surprise that right is listed on the same side as light, good, male, straight, etc., while left is listed alongside darkness, evil, female, crooked, etc. [12]. Similarly, the early Greek philosopher Anaxagoras thought that only the sperm from the right testicle produced boys [13].

The reason for ancient Greeks' consideration of left-handedness as evil work and a bad sign may be found in the myth of the castration of Uranus. Cyclops and Titans were the sons of Gaia (Mother Earth) and Uranus. After their birth, Uranus closed Cyclops to the underworld (Tartar). Eager for revenge, Gaia invited Titans to punish their father. While Uranus was making love with Gaia, the youngest Titan, named Cronus, grabbed Uranus's genitals with the left hand and cut them off by a stone sickle [14].

One interesting investigation examines the data on scrotal asymmetry in some detail, and puts them in the context of Greek theories of functional differences between the right side and the left side [15]. Famous is the ancient debate between Plato and his student Aristotle about left-handedness. Plato, a right-hander, said that the dominance of hand skills was learned, while left-handed Aristotle in his book *Metaphysics* claimed that people were naturally right-handed or left-handed [16].

Ancient Romans were also very pro-right-handedness. For example, they claimed that wearing a wedding ring on the third finger of the left hand would fend off evil that is in the left-hand. Shaking right hands in greeting dates back to ancient Rome as a proof of the absence of hidden weapons. It seems that Julius Caesar encouraged this ritual being permanently afraid of assassination [17].

Surprisingly, Yin in ancient China is associated with female sex, darkness, and right-handedness, while Yang is related to masculinity and light, but also to left side. In spite of that, modern China advocates the domination of right-handedness [12].

Ancient Incas believed that left-handed individuals had a magic power of healing people. Eskimos also believed left-handed individuals to be wizards [13].

RECENT HISTORY

During the Middle Ages, left-handedness was considered "sinful." Under the strong influence of the Catholic Church, left-handedness was connected with the "devil," "weakness," "feminine," "unhealthy," "filthy," with something that had to be forced to turn to the "good-right" side [18].

Left-handed people were accused of friendship with the devil. During the Inquisition, left-handedness was sufficient to sentence a woman to death as a witch. Many innocent left-handed people were executed in this way [17]. The right arm was blessed, while the left-handed served the devil. Making a sign of the cross with left hand was a heresy that caused hell. An analysis concerning handedness in the Bible found about 100 positive references to right and the right arm and about 25 negative references to left and the left hand. A similar point of view can be found in the Qur'an. In both Islam and Hinduism, the right hand is used exclusively during solemn ceremonies [10].

In contrast, there are some minor religions, like Tantric Buddhism for example, in which left-handedness was considered positive, and a symbol of wisdom [12, 17].

In addition to religion, occult sciences, such as Tarot cards, engage in prejudices on left-handedness. Two of the most famous tarot cards, Lady Justice and the Devil, symbolically depict such prejudices. Lady Justice is holding a sword in her right hand, while the devil uses his left hand [12, 18].

During the Renaissance, the interest for nature and human beings flourished. It is reported that Michelangelo and Leonardo da Vinci used both hands in their works [19]. One study suggests that the handedness of Leonardo da Vinci is controversial. There is no doubt that many of his well-known drawings were drawn with the left hand, and there are some indications that he may have become left-handed as the result of an injury to his right hand in early adulthood [20]. The handedness of Michelangelo Buonarroti, is also controversial. Although there is no doubt that almost all of his drawings were drawn with the right hand, an unfairly unknown autobiography of Raffaello da Montelupo stated that Michelangelo, a natural lefty, trained himself from a young age to become right-handed. This biography also underlined that Michelangelo restricted the use of his left hand only to actions requiring force, such as hammering, carving, and chiseling marble [21].

Unfortunately, in the 18th and 19th centuries, the discrimination against left-handed people was strong and institutionalized. It included such practices as tying a child's left hand behind his chair or corporal punishment for anyone caught writing with the left hand. During the industrial revolution, left-handedness was particularly unfavorable, as machines and tools were designed for the right-handed people [17].

In the 19th century, Cesare Lombroso, an Italian criminologist and physician, connected left-handedness with savagery and crime. Fortunately, his ideas on left-handedness and human behaviour were later discarded [22].

Very important for understanding handedness was a famous discovery of French scientist Broca (Pierre Paul

Broca) about the asymmetry of brain hemispheres, in 1860. After Broca's investigations, the interest in studying laterality declined for a while, and was renewed exactly one century later, in 1960. By studying the patients who underwent an operation of severing a commissure that connects the left and the right hemisphere of the brain in order to control intractable epilepsy, it was concluded that the left hemisphere is specialized for language, and the right one for emotional and nonverbal functions. This research brought Roger W. Sperry a Nobel Prize in Physiology or Medicine in 1981 [16].

Despite these epochal discoveries, the practice of discrimination against left-handed people continued in the 20th century. Even in scientific circles, there were opinions that handedness was a result of a certain pathology. For example, in the research by Gordon [23], an increased number of left-handed people was found among the mentally retarded.

In the mid-20th century, Abram Blau, an American psychoanalyst and child psychiatrist, was still suggesting that left-handedness was merely due to perversity and the result of emotional negativism in childhood. Blau claimed that left-handed people became stubborn, rebellious, rigid, and for some reason obsessed with cleanliness in adulthood. An influential British educational psychologist Cyril Burt supported Blau's ideas, describing left-handed people as "stubborn and willful," as well as "awkward" and "clumsy" [9].

It is interesting to mention an article in the daily newspaper *Pravda* (Kingdom of Serbs, Croats, and Slovenes) of April 6, 1937 about a "Left-handed Club" in New York founded by a Methodist priest. He was also left-handed and he shared blessings using his left hand. In this club, he gave lectures where he claimed that left-handedness was a great gift of nature and that left-handed people were more able and talented than right-handed people. He supported this attitude by the fact that many great people were left-handed. The number of members of this club was unusually large [24].

North America is liberal towards left-handedness, as evidenced by a higher percentage of left-handed people compared to the rest of the world, because socio-cultural pressure was less frequent. Even in 1933, in the daily newspaper *Pravda* in the "Small Medical Curiosities," there was a short article titled "A Disappearing Left-handedness." It stated that there was a large number of children who were left-handed in childhood, but later became "normal" and regularly used the right hand. "According to the latest statistics," the paper stated, "in Europe there are about 10% of left-handed children, and only 2% of left-handed adults" [25]. This difference could be explained by a socio-cultural pressure against left-handed people of this age. Such a drastic reduction in the presence of left-handed people some researchers today explain by socio-cultural pressure against left-handed people, while others advocate the hypothesis of a shortened lifespan of left-handed people in a right-handed-designed world [26]. In the Conway's handbook published in 1935, *The Prevention and Correction of Left-Handedness in Children*, he recommended training children from infancy to overcome left-handedness, which came as a result of parental "indifference to the seriousness of the handicap," which was a "sinisitral condition," a "disease" that needed to be recognized along the same lines as "rickets, pneumonia and colic" [27].

In the 1960s and 1970s, Catholic schools continued with psychological pressures against left-handed children, and sometimes also retained corporal punishment. Until the end of the 1970s in the countries of the Soviet Bloc as well as in former Yugoslavia, there were similar practices against left-handed people. In Albania, left-handedness was declared illegal and was punishable as a crime, while in Japan left-handedness of a woman was grounds for divorce [28].

LEFT-HANDEDNESS TODAY

Social tolerance was officially accepted for left-handed people in the 21st century, but it is not uniform worldwide. Even in a relatively open-minded and informed society of today, parents and teachers may encourage a left-handed child to switch to right-handedness to make their lives easier in a largely right-handed world. A good percentage of natural left-handed people tell of their own self-inflicted attempts to switch hands during childhood [29].

This pressure to change the dominant hand may cause huge stress during childhood and education. A vulnerable group that should be put in focus for the prevention during the school period are left-handed students in all fields, particularly those of longer and demanding study programs. Studies show that examination process and prolonged studies present an additional risk factor for stressful experiences in students, such as students of medicine [30].

At the level of social cognition in Serbia, subtle prejudice against this minority group is still present and visible. This is demonstrated by the fact that the prevalence of left-handedness is lower in Serbia than in Western Europe (5–10% *vs*. 11–14%) [29]. In a study conducted in Belgrade on a sample of 1,189 children aged 15–19 years, the percentage of left-handed children was 6.8%, with a significantly higher number of male left-handed people compared to female population (8.9% *vs*. 4.8%) [26].

Nowadays, left-handedness is considered to be a special gift, and left-handed people have started to evaluate themselves differently, even favouring themselves as more competent in relation to the right-handed people. "The last neglected minority" has begun to organize themselves on websites such as www.anythinglefthanded.co.uk. The August 13th has been declared the International Day of Left-handed People. Left-handed people are slowly becoming proud of their former "handicap." A list of left-handed individuals who have marked the human history has become very long and includes writers (Honoré de Balzac, Mark Twain, Charles Dickens, Lois Carroll, Franz Kafka, Gabriel García Márquez), musicians (Ludwig Van Beethoven, Wolfgang Amadeus Mozart, Sergei Prokofiev, Sergei Rachmaninoff, Maurice Ravel, Robert Schumann, Niccolo Paganini, Ringo Starr, George Michael, Sir Paul McCartney, Jimi Hendrix, Bob Dylan, Phil Collins), artists (Michelangelo Buonaroti, Leonardo da Vinci, Lautrec de Toulouse, Peter Paul Rubens), actors (Greta Garbo, Judy Garland, Fred Astaire, Charlie Chaplin, Marilyn Monroe,

Rock Hudson, Anthony Perkins, Pierce Brosnan, Oprah Winfrey, Julia Roberts, Sarah Jessica Parker, Demi Moore, Nicole Kidman, Kim Basinger, Sylvester Stallone), philosophers-scientists (Aristotle, Friedrich Nietzsche, Albert Einstein, Ivan Pavlov, Marie Curie, Nikola Tesla), athletes (Valentino Rossi, Diego Maradona, Goran Ivanišević, John McEnroe, Martina Navratilova, Monica Seles, Rafael Nadal), leaders (Ramses II, Tiberius, Julius Caesar, Alexander the Great, Napoleon Bonaparte) [31].

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Историјски аспекти леворукости

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САЖЕТАК

Латерализација је једно од централних питања у неурологији, неуропсихологији и другим сродним научним дисциплинама. Постоје незнатне промене у пропорцији леворуких од млађег палеолитног доба пре 10.000 година и процењена је на око 10%. Како се историја људског размишљања развијала од сујеверја према науци, објашњење за леворукост променило се од „ђаволјег посла" до неуролошке специфичности. Овај рад приказује ову веома интересантну историјску промену анализирајући податке о леворукости и ставовима према њој у људском друштву од праисторије до данас. Чак и у релативно отвореним друштвима родитељи и наставници могу охрабривати леворуко дете да се превежба на деснорукост, да би им живот учинили лакшим у већински десноруком свету. С друге стране, на леворукост се све више гледа као на посебан дар, а леворуки су почели себи да дају предност и већу компетентност у односу на деснораке људе.

Кључне речи: латерализација; доминантност руке; историја

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 СА 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:
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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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AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

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.

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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.

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles, review article and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article includes: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mm Hg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

LENGTH OF PAPER. The entire text of the manuscript - title page, summary, the whole text, list of references, all

enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian - must not exceed 5,000 words for original articles, review articles and articles on history of medicine, and 3,000 words for case reports, preliminary and short communications, current topics, articles for practitioners, educational articles and articles for "Language of medicine", congress and scientific meeting reports; for any other section maximum is 1,500 words.

Video-articles are to last 5-7 minutes and need to be submitted in the flv video format. The first shot of the video must contain the following: title of the journal in the heading (*Serbian Archives of Medicine*), title of the work, last names and initials of first and middle names of the paper's authors (not those of the creators of the video), year of creation. The second shot must show summary of the paper, up to 350 words long. The final shot of the video may list technical staff (director, cameraman, lighting, sound, photography, etc.). Video-articles need to be submitted along with a separate summary (up to 350 words), a single still/photograph as an illustration of the video, and a statement signed by the technical staff renouncing copyrights in favor of the paper's authors. To check the required number of words in the manuscript, please use the menu *Tools-Word Count*, or *File-Properties-Statistics*.

ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

TABLES. Each table, with its legend, should be self-explanatory. The title should be typed above the table and any explanatory information under the table. Tables should be numbered in Arabic numerals in order of citation in the text. Use *MS Word*, the menu *Table-Insert-Table*, inserting the adequate number of rows and columns. By the right click of the mouse, use the options *Merge Cells* and *Split Cells*. Use *Times New Roman*, font size 12 pt, with single line spacing and no indent to draw tables. Abbreviations used in tables should be explained in the legend below each respective table.

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