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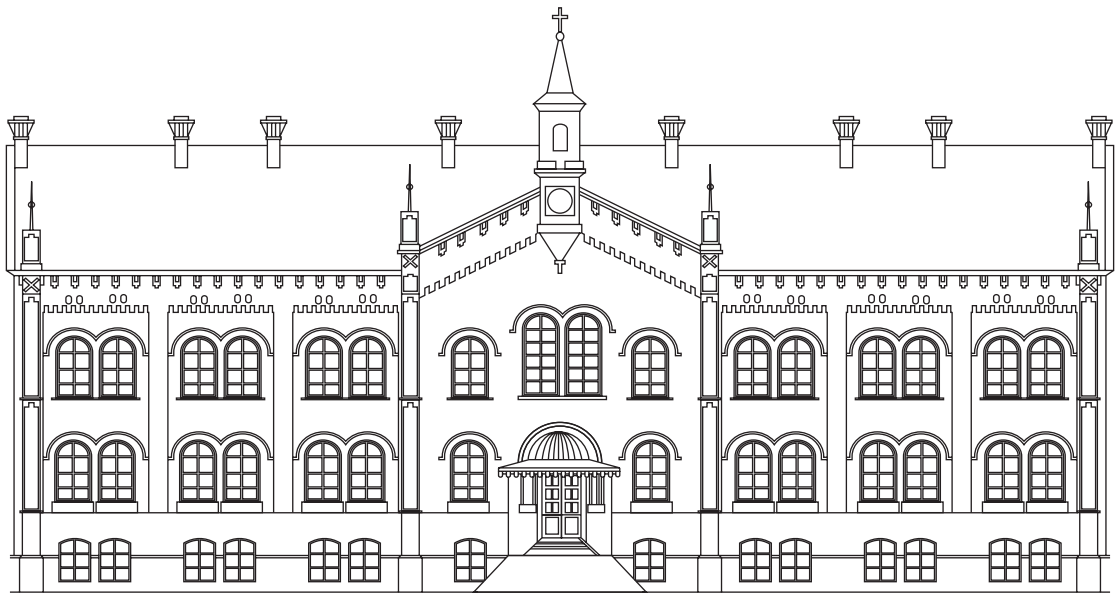

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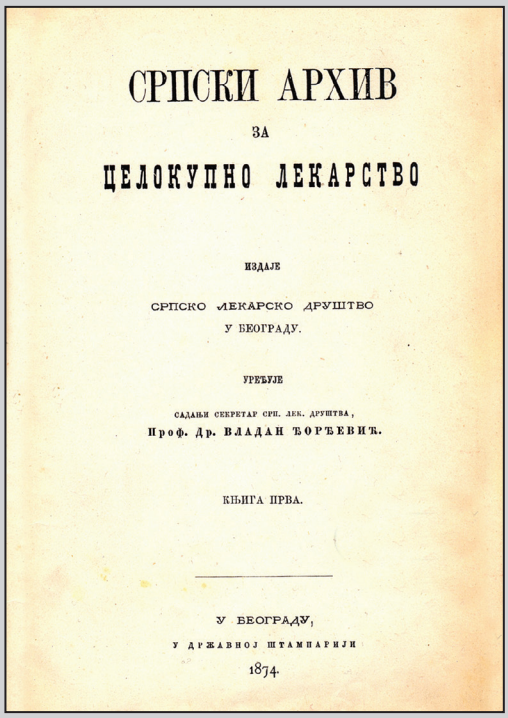

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

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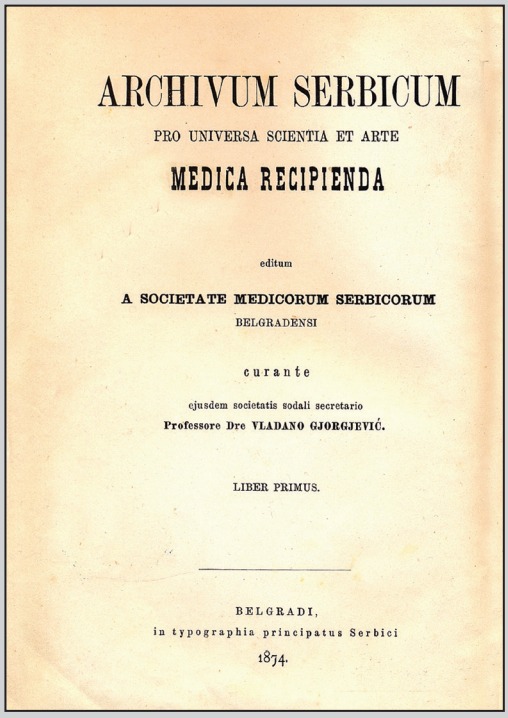
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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1874.

The title page of the first journal volume in Latin

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REVIEW ARTICLE • ПРЕГЛЕД ЛИТЕРАТУРЕ

Editorial

Српско лекарско друштво одаје последњу почаст свим својим члановима, докторима, медицинском и немедицинском особљу широм света палом у борби са пандемијом COVID-19. Изрази саучешћа породицама преминулих пацијената.

Нека им је вечна слава и хвала!

The Serbian Medical Society pays its respects to all its members, doctors, nurses, and allied medical and non-medical personnel together with all the patients worldwide who had lost the battle against the COVID-19 pandemic.

May they all rest in peace!

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The diabetic dental pulp repair – involvement of vascular endothelial growth factor and bone morphogenetic protein 2

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SUMMARY

Introduction/Objective We aimed to investigate the effects of diabetes mellitus (DM) on rat dental pulp repair by measuring time-dependent changes in expressions of vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP 2) following direct pulp capping.

Methods Two groups, each of 20 Wistar rats, received either streptozotocin (for DM induction) or the same volume of sterile saline. A week later, the pulp of maxillary and mandibular right incisors in diabetic and non-diabetic groups were exposed and capped with calcium hydroxide in order to provoke reparative response. The levels of VEGF and BMP 2 were determined in the pulp tissue lysates one and seven days after the pulp capping, using enzyme-linked immunosorbent assays.

Results Diabetic state *per se* increased VEGF level, with a peak at first day after the pulp capping (19.3 ± 0.9 pg/mg, $p < 0.001$), but did not affect BMP 2 levels. Significant increase of BMP 2 expression was noticed on the seventh day in capped pulp, but only in diabetic rats (16.7 ± 1 pg/mg, $p = 0.001$). Positive correlation between VEGF and BMP 2 was found on the seventh day following capping, only in diabetic pulp ($r = 0.905$, $p = 0.003$).

Conclusion Diabetes-induced increase in VEGF expression reflects changes in the inflammatory phase of pulp repair in DM. Increase in BMP 2 expression suggest that stimulating effect of calcium hydroxide appears seven days after diabetic pulp capping.

Keywords: dental pulp capping; diabetes; vascular endothelial growth factor; bone morphogenetic protein 2; calcium hydroxide

INTRODUCTION

Under pathological conditions, such as injury or infection, the dentine–pulp complex shows significant reparative response initiated by an inflammatory reaction, prerequisite for pulp healing and mediated principally by macrophages [1]. Besides clearing the injury site, macrophages are the main source of the growth factors (GF), including vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP 2), required for tissue repair [2, 3].

Dental pulp repair rely on dental pulp stem cells that migrate to the site of injury, differentiate and proliferate into endothelial cells, involved in angiogenesis, or into odontoblasts – for dentin generation, processes regulated by GF, among others VEGF and BMP 2 [1, 4]. Recent study by Aksel et al. [4] showed that *in vitro* delivery of VEGF and BMP 2 significantly enhanced the angiogenic and odontogenic potential of human dental pulp stem cells. Noteworthy, the alteration in expression of VEGF and BMP 2 has been identified within human dental pulp cells in inflammation as well as in human diabetic pulp tissue [5, 6, 7].

Diabetes mellitus (DM) impedes the healing of dental pulp resulting in inadequate reparative response, yet underlying molecular mechanisms are still not clarified [8]. A recent study investigating diabetic wound healing point at alterations in the inflammatory phase due to macrophages dysfunction as a critical event in impaired tissue healing in DM [9]. Having in mind that the dental pulp, due to its limited collateral circulation, is especially sensitive to diabetes-induced circulatory disorder and associated failure to deliver components of the immune system and GF, we aimed to investigate effects of diabetes on initial events of dental pulp repair by means of measuring time-dependent changes in VEGF and BMP 2 expression in rat dental pulp following direct capping [10].

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METHODS

Reagents

Streptozotocin (STZ) was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The enzyme-linked immunosorbent assay (ELISA) kits for VEGF (Rat VEGF ELISA Kit) were purchased from RayBiotech Inc. (RayBiotech Inc., Norcross, GA, USA) and the ELISA kits for BMP 2 (Quantikine BMP-2 Immunoassay) were purchased from R&D Systems Inc. (R&D Systems Inc., Minneapolis, MN, USA). Other reagents, medicaments, and dental materials were procured from standard local commercial suppliers.

Experimental animals

The study was conducted on 40 Wistar rats with a body weight between 250 g and 300 g obtained from the Military Medical Academy in Belgrade, Serbia. The study was reviewed and approved by the Ethics committee of the School of Dental Medicine, University of Belgrade (approval number: 36/8) and was carried out in accordance with the EU Directive 2010/63/EU for animal experiments. The animals were randomly allocated in either experimental (diabetic) or control (non-diabetic) group (20 animals per group). Both the experimental and the control group were additionally divided into two groups of 10 animals according to the duration of induced pulp reparative response (day one and day seven). All rats were housed in wire-bottomed cages (five animals per cage), with *ad libitum* food and water, on a 12-hour light-dark schedule.

Diabetes induction

All the animals underwent overnight fasting prior to induction of hyperglycemia by intraperitoneal injection of 60 mg/kg of STZ, *ex tempore* dissolved in sterile saline [11]. Animals in control group were injected with the same volume of sterile saline. Blood glucose levels were estimated on blood from tail vein using GlucoSure glucometer and Touch-In test strips (Apex Biotechnology Corp, Taiwan), five minutes before, 24 hours, and seven days after STZ or saline administration. Only the animals showing 200 mg/dl blood glucose level were considered as diabetic.

Operative procedures

One week after the STZ or sterile saline injection, all animals underwent cavity preparation procedures on distal surfaces of right mandibular and maxillary incisors in order to provoke pulp reparative response. Left incisors remained intact and served as controls. The rats were anaesthetized with an intramuscular injection of 20 mg/kg tiletamine-zolazepam combination (Zoletil 100, Virbac, Carros, France). Before cavity preparation, the oral cavity was disinfected with 0.2% chlorhexidine digluconate (Curasept 220, Curaden International AG, Kriens, Switzerland) and teeth additionally scrubbed with cotton pellet soaked with 70% ethanol. Aided with magnifying glasses (magnification 4.5×; Zeiss, Aalen, Germany), cavities were prepared with a micro motor handpiece and a carbide round burs (ISO 006; NTI, Kahla, Germany) until the pulp was visible. The cavities were prepared under constant water cooling. Pulp exposure was subsequently created with a sterile sharp probe (Ref. 27-3; HLW, Wernberg-Köblitz, Germany). The cavities were rinsed with saline solution, and hemostasis obtained with sterile, saline soaked paper points. After the careful air-drying of the cavities, pulp tissue was directly capped with calcium hydroxide ($Ca(OH)_2$) paste (Life; Kerr Corp., Orange, CA, USA) in order to induce reparative response, and cavities were restored using a self-etch, flowable composite restoration material (Vertise Flow; Kerr Corp., Orange, CA, USA).

Sample collection and preparation

Sacrifices of randomly chosen 10 animals in both diabetic and non-diabetic group were done one day after the capping procedure, using an overdose of thiopental-Na (Trapanal, Nycomed, Konstanz, Germany). The sacrifices of remaining 10 animals in diabetic and non-diabetic groups were done in the same way seven days after the induction of pulp reparation. The incisor teeth were extracted and split using excavators and pliers, and pulp tissue evacuated with sterile probes and tweezers. The samples of pulp in reparation were formed as pools of pulp tissue from right mandibular and maxillary incisors of a single animal. Similarly, pools of intact pulp tissue were obtained from contra lateral intact incisors. Specimens were transferred directly to previously weighed Eppendorf tubes. Tubes with pulp tissue were then measured for total weight and stored at -70°C until further use. All weight measurements were conducted using high precision (readability: 10^{-4} g) Adventurer ™ digital balance (OHAUS, Corp., Pine Brook, NJ, USA). The samples weights were calculated by subtracting total and empty tubes weights.

After homogenization, the pulp tissue lysates were centrifuged at 5000 g for 10 minutes in micro-centrifuge (Heraeus* Biofuge Primo R, Thermo Fisher Scientific, Waltham, MA, USA), the supernatants were collected, divided into two aliquots (for VEGF and BMP 2 concentration measurement) and stored at -70°C until further analysis.

Vascular endothelial growth factor and bone morphogenetic protein 2 quantification

Concentrations of VEGF and BMP 2 were measured in supernatants of rat pulp tissue lysates using ELISA kits according to the manufacturer's instructions. The absorbencies of microplate wells at 450 nm were recorded using Multiskan EX microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Each standard and sample was run in duplicate for both GF and the value used for statistical analysis was the average of two readings. The final GF concentrations of each pulp sample were normalized to its total weight. Results were expressed as pg/mg of pulp tissue.

Statistical analysis

Mean values and standard error of mean (SEM) were used for descriptive statistics of the sample. Parametric statistical analysis was used since the obtained concentrations of GF, blood glucose levels and body weight were normally distributed (Shapiro-Wilk test, $p > 0.05$) and variance of observed groups were homogenous ($p > 0.05$). Data were analyzed using one-way analysis of variance (ANOVA) with *post hoc* Holm-Sidak method for pairwise comparisons, and Pearson correlation coefficient. Analyses were computed with the statistical software SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). A p value less than 0.05 was considered to be statistically significant.

RESULTS

Variables of diabetic state

Diabetic animals expressed hyperglycemia and significant loss of body weight (Table 1).

Table 1. Glycaemia (mg/dL) and body weight (g) values in experimental animals

Group	Initial glycaemia	Final glycaemia	Initial weight	Final weight
Diabetic	90.8 ± 2	204.7 ± 2.3[a]	277 ± 5.78[c]	246 ± 6.18[d]
Non-diabetic	95.1 ± 2.3	100.9 ± 2.2[b]	287 ± 4.96	299 ± 3.7

[a]*vs.* glycaemia in all other groups;
[b]*vs.* initial glycaemia in diabetic group;
[c]*vs.* final body weight in diabetic group;
[d]*vs.* body weight in nondiabetic groups;
Values are presented as mean ± standard error of mean. Superscript letters represent significant differences ($p < 0.05$, One-way ANOVA & Holm-Sidak post hoc test).
Diabetic group – experimental animals received streptozotocin; Non-diabetic group – experimental animals received saline; initial glycaemia and body weight values were estimated five minutes before Streptozotocin/saline injection; final glycaemia and body weight values were estimated at the time of sacrifice;

Vascular endothelial growth factor protein expression in dental pulp

Diabetic rats showed significant increase of the VEGF pulp levels ($p < 0.001$) compared to non-diabetic in both intact and capped pulp, regardless of examined capping time duration (Figure 1). Diabetes *per se* increased VEGF levels at both time points: 19.3 ± 0.9 pg/mg in diabetic *vs.* 11.7 ± 1.8 pg/mg in non-diabetic intact pulp on the first day, and 18.5 ± 0.6 pg/mg in diabetic *vs* 10.7 ± 1.2 pg/mg in non-diabetic intact pulp on the seventh day. Also, capping with $Ca(OH)_2$ *per se* increased VEGF levels on the first day both in diabetic pulp (28.2 ± 1.7 pg/mg in capped *vs.* 19.3 ± 0.9 pg/mg in intact pulp) and in non-diabetic (18.9 ± 1.0 pg/mg in capped *vs.* 11.7 ± 1.8 pg/mg in intact pulp).

Bone morphogenetic protein 2 protein expression in dental pulp

BMP 2 pulp levels were not significantly altered in diabetic compared to non-diabetic animals, in either intact or capped pulp. However, on the seventh day, diabetic capped pulp showed significantly higher BMP 2 levels compared to

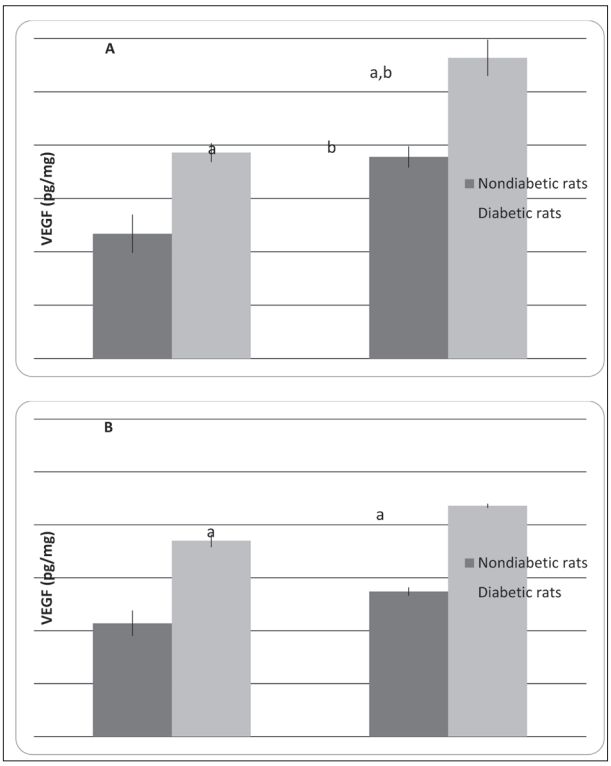

Figure 1. Vascular endothelial growth factor levels (pg/mg of tissue) in intact and capped dental pulp in diabetic and nondiabetic rat. Bars represent mean ± standard error of mean of pulp vascular endothelial growth factor levels on the first (A) and the seventh day (B) after pulp capping in diabetic and nondiabetic rat
[a]$p < 0.001$ diabetic compared to nondiabetic rats;
[b]$p < 0.001$ capped compared to intact pulp

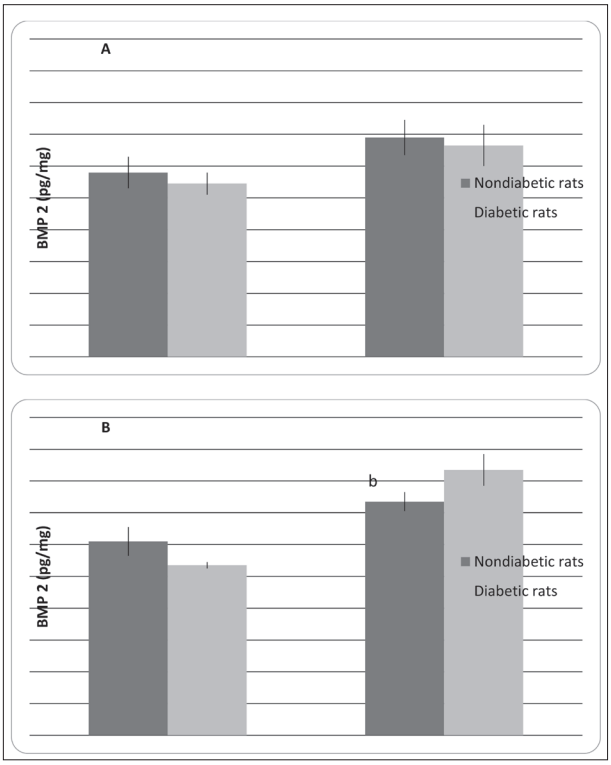

Figure 2. Bone morphogenetic protein 2 pulp concentrations (pg/mg of tissue) in intact and capped dental pulp in diabetic and nondiabetic rat. Bars represent mean ± standard error of mean of the pulp bone morphogenetic protein 2 levels on first (A) and seventh day (B) after pulp capping in diabetic and nondiabetic rat
b – $p = 0.001$ capped compared to intact pulp

Table 2. Vascular endothelial growth factor and bone morphogenetic protein 2 correlations

Experimental groups		I	II	III	IV	V	VI	VII	VIII
	GF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF
I	BMP 2	r = -0.353 p = 0.560							
II	BMP 2		r = -0.248 p = 0.688						
III	BMP 2			r = 0.905* p = 0.0348					
IV	BMP 2				r = -0.410 p = 0.493				
V	BMP 2					r = -0.881* p = 0.0482			
VI	BMP 2						r = 0.592 p = 0.293		
VII	BMP 2							r = -0.363 p = 0.548	
VIII	BMP 2								r = -0.662 p = 0.224

GF – growth factor; r – Pearson correlation coefficient;
*significant correlation ($p < 0.05$); experimental groups: I – diabetic rats, capped pulp, first day; II – diabetic rats, intact pulp, first day; III – diabetic rats, capped pulp, seventh day; IV – diabetic rats, intact pulp, seventh day; V – non-diabetic rats, capped pulp, first day; VI – non-diabetic rats, intact pulp, first day; VII – non-diabetic rats, capped pulp, seventh day; VIII – non-diabetic rats, intact pulp, seventh day;

diabetic intact pulp (16.7 ± 1.0 pg/mg *vs*. 10.7 ± 0.2 pg/mg, p = 0.001) (Figure 2).

Vascular endothelial growth factor - bone morphogenetic protein 2 correlations

In non-diabetic animals, there was a significant negative correlation between pulp VEGF and BMP 2 levels on the first day after capping. In diabetic pulp samples, VEGF and BMP 2 were positively correlated on the seventh day after capping (Table 2).

DISCUSSION

Pulp healing in rats shows histological similarity to pulp healing in humans after direct pulp capping with different dentinogenetic-stimulating agents [12]. Although rat molar teeth are more frequently used as model for human teeth for histological analysis of dentin repair [13], in the present study, due to voluminous pulp, incisor teeth were used in order to provide the required amount of pulp tissue necessary for VEGF and BMP 2 quantification. Rodent incisor teeth, although differing from human for their constant growth, were proposed as a useful model for evaluating potential human dental pulp reactions to pulp capping agents and were used previously to evaluate several aspects of pulp repair and inflammation [14, 15]. The repair of dental pulp after direct capping by $Ca(OH)_2$ implies following sequential steps: a moderate inflammation, migration of dental pulp stem cells, their proliferation and differentiation resulting in reparatory dentin formation [1]. Inflammation is prerequisite for pulp repair as a first step, resolved during the first week and is characterized by infiltration of macrophages starting from the first day after trauma or capping [1, 16]. Spiller et al. [17] showed that M1 macrophages, predominant in initial inflammatory phase, express VEGF gene in order to support angiogenesis but also, VEGF is necessary for M1 to shift to M2 macrophage phenotype and to resolve inflammation [18].

Present results show that capping procedure induce VEGF expression being the prominent on the first day after the procedure while returning to control levels on the seventh day after the capping, suggesting significance of VEGF for the inflammatory phase of pulp repair after capping. Namely, previous studies showed an increase in capillary proliferation and inflammation being intensive one to three days after direct pulp capping of rat teeth with calcium hydroxide, suggesting time line for inflammatory phase following capping [12].

The effects of $Ca(OH)_2$ as capping material rely on its dissociation into calcium and hydroxyl ions. Hydroxyl ions exhibit antibacterial properties due to alkaline reaction and stimulates reparative dentin formation [19]. Oxidation of hydroxyl ions results in formation of hydroxyl radicals, which is able, as reactive oxygen species (ROS), to induce VEGF expression [20]. Beside ROS, another potent stimulus for VEGF induction is hypoxia. In line with this, our results show that experimentally induced diabetes caused a significant increase of VEGF levels in intact and capped dental pulp. This is probably induced by both hyperglycemia and hypoxia, strong stimuli for VEGF induction, effects potentiated by the fact that dental pulp has limited or no collateral circulation, therefore is more prone to hypoxia-induced VEGF induction [21]. Furthermore, intensive ROS generation and resulting oxidative stress are hallmark of diabetes and induced VEGF [22] and, accordingly, our previous studies showed oxidative stress in dental pulp tissue of patients with type 2 DM [23]. In line with the proposed mechanism of redox-mediated VEGF induction by $Ca(OH)_2$ and DM is the fact that present

results show the greatest VEGF induction in both capped and diabetic dental pulp. Having in mind that diabetic state is characterized by an imbalance in the ratio of M2-"anti-inflammatory" and M1-"pro-inflammatory" macrophages in the favor of the latter [24], present results of enhanced VEGF expression in DM could be reflecting proinflammatory state induced by M1 macrophages prevalence, but also VEGF overexpression could contribute to resolving inflammation by activation of M2 "anti-inflammatory macrophages".

Regarding BMP-2 levels, present results show significant increase in BMP 2 expression only in diabetic rats, seven days after pulp capping, suggesting that BMP-2 induction depends on both $Ca(OH)_2$ and diabetic state. It is well known that BMP 2 promotes the differentiation of pulp stem cells into odontoblasts and production of reparative dentin, phases following inflammation resolution [25] which is in line with presently observed negative correlation between VEGF and BMP-2 observed on the first day after capping in non-diabetic pulps. Regarding mechanisms underlying stimulatory effects of calcium hydroxide on BMP 2 expression under diabetic state, it is noteworthy that in addition to ROS-stimulated BMP 2 expression [26], increased availability of Ca^{+2} ions is also associated with an increase in cellular BMP 2 expression [27]. These effects are potentiated in the diabetic state- state of oxidative stress and acidic environment which enhance BMP 2 expression and stability [28] but also, BMP 2 -VEGF mutual association, suggested by observed positive correlation between BMP-2 and VEGF in DM. Having in mind that dental pulp stem cells isolated from diabetic patients show diminished capacity for proliferation and differentiation and that BMP 2 supplementation enhanced differentiation of pulp stem cells into odontoblasts *in vitro* [29], present results point at the beneficial effects of calcium hydroxide as the direct capping agent in the DM for pulp repair.

CONCLUSION

Studies of dental pulp repair processes *in vivo* in humans are ethically and practically limited and, therefore, present results obtained in diabetic rat represent biologic background for consideration of therapies directed toward maintaining pulp vitality in diabetic dental pulp. Namely, we showed DM and calcium hydroxide induced increase in VEGF expression, which reflects changes in the inflammatory phase of pulp healing. On the other side, our results point to the beneficial effects of $Ca(OH)_2$ in direct capping in DM due to an increase in BMP 2 – the critical mediator for reparative dentin formation.

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Репарација дијабетичне зубне пулпе – улога васкуларног ендотелног фактора раста и костног морфогенетског протеина 2

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да се испита ефекат дијабетеса мелитуса на репарацију зубне пулпе пацова утврђивањем временски зависних промена у експресији васкуларног ендотелног фактора раста (*VEGF*) и костног морфогенетског протеина 2 (*BMP* 2) после директног прекривања пулпе.

Методе Истраживање је спроведено на пацовима соја вистар, подељеним у две групе од по 20 животиња, при чему је једна група добила стрептозотоцин (за индукцију дијабетеса мелитуса), а друга стерилни физиолошки раствор у истој запремини. После недељу дана пулпе максиларних и мандибуларних дољих инцизива код дијабетичних и недијабетичних животиња су експониране и одмах затим прекривене калцијум-хидроксидом да би се изазвао репараторни одговор. Нивои *VEGF* и *BMP* 2 су утврђивани у лизатима пулпног ткива, првог и седмог дана после директног прекривања имуноензимским тестом *ELISA*.

Резултати Дијабетично стање је довело до раста нивоа *VEGF*, са максимумом утврђеним првог дана после прекривања пулпе (19,3 ± 0,9 *pg/mg*, $p < 0,001$), али није утицало на нивое *BMP* 2. Значајан пораст *BMP* 2 је утврђен седмог дана после прекривања пулпе, али само код дијабетичних пацова (16,7 ± 1 *pg/mg*, $p = 0,001$). Код ових животиња у истом периоду после прекривања нађена је позитивна корелација између нивоа *VEGF* и *BMP* 2 ($r = 0,905$, $p = 0,003$).

Закључак Дијабетесом индукован пораст експресије *VEGF* указује на промене у инфламаторној фази пулпне репарације. Пораст експресије *BMP* 2 указује да се стимулативан репараторни ефекат калцијум-хидроксида јавља седмог дана после прекривања дијабетичне пулпе.

Кључне речи: прекривање пулпе; дијабетес; васкуларни ендотелни фактор раста; костни морфогенетски протеин 2; калцијум-хидроксид

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Microcomputed tomography cortical bone evaluation for craniofacial implantology

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SUMMARY
Introduction Good implant stability is one of the most important factors for successful implant therapy. This precondition is important for all kinds of implants, oral and extra-oral, i.e. craniofacial implants as well. One of the most important factors for satisfactory implant stability is the bone quality, particularly of the cortical bone, which is determined by its microarchitectural parameters.
The aim of this paper was to assess cortical bone microarchitectural parameters in the targeted regions for craniofacial implant placement.
Methods Bone quality on targeted locations was determined by the micro-CT method on a cadaver model. The target places for implant placement were the periorbital, the perinasal, and the periauricular region. Microarchitectural parameters included cortical thickness (Ct.Th.), cortical porosity (Ct.Po.), pore diameter (Po.Dm.), and pore separation (Po.Sp.).
Results The smallest Ct.Po. (4.1%) and the largest Po.Sp. (0.5 mm) were determined in glabella. The maximum Ct.Th. (2.7 mm) as well as Po.Dm. (0.2 mm) were found in the zygomatic region. The mastoid part of the temporal bone showed the smallest Ct.Th. (1.2 mm) and Po.Sp. (0.3 mm). The highest Ct.Po. was in the perinasal region (8.5%).
Conclusion The bone quality measured through microarchitectural parameters was good in all the regions of interest for the disk- and screw-shape extra-oral implant anchorage.
Keywords: microarchitecture; bone quality; micro-CT

INTRODUCTION

Patients with different facial defects (orbital, nasal, auricular) are indicated for craniofacial implant therapy and prosthetic rehabilitation. Majority of them have undergone previous tumor resections, which could cause the lack of the bone needed for implant placement. Good implant stability is important for stable maxillofacial prosthesis anchorage [1, 2]. One of the most important factors for successful implant therapy is the bone quality and quantity [3]. For this reason, implant therapy should be well planned and carefully carried out. For craniofacial implant stabilization, microarchitectural parameters of cortical bone in the targeted implant placement points are particularly important. [2, 3]. The periorbital, the perinasal, and the periauricular region, which are used for implant anchorage, have different bone microstructure, which could affect the final outcome of the implant therapy [3]. Microtomography (micro-computed tomography – micro-CT) is a method to image and quantify bone tissue. It has the capability to assess the architecture and the mechanical properties of the bone [4].

The aim of this paper was to assess cortical bone microarchitectural parameters in the targeted regions for craniofacial implant placement.

METHODS

The research was performed at the Laboratory for Anthropology, Institute of Anatomy, School of Medicine, University of Belgrade. The study was reviewed and approved by the Committee on Ethics of the School of Dental Medicine, University of Belgrade (No. 36/14).

A young Caucasian adult's dry scull from the collection of the Laboratory for Anthropology, Faculty of Medicine, University of Belgrade, was selected in order to perform the micro-CT analysis of the targeted implant placement areas and to evaluate the microarchitectural parameters which define the quality of the cortical bone. Sexual and demographic characteristics were moderately expressed, thus the skull used presented an average anatomical sample for the situation.

According to the implant placement points for maxillofacial prosthetic rehabilitation, the following locations were selected: for nasal implants – glabellar part of the frontal bone and lateral walls of the nasal pyramid; for orbital implants – upper and lower (cranial and caudal) lateral edges of the orbit and the body of the zygomatic bone; for auricular implants – the petrous part of the temporal bone (Figure 1).

Based on these targeted implant placement points, the following areas for micro-CT scanning were selected: supraorbital margin – orbit, body of the zygoma, glabella, mastoid process, piriform aperture.

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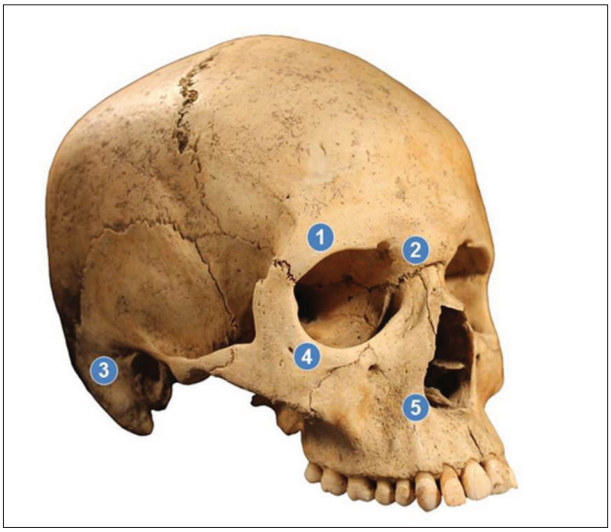

Figure 1. Micro-computed tomography scanning areas

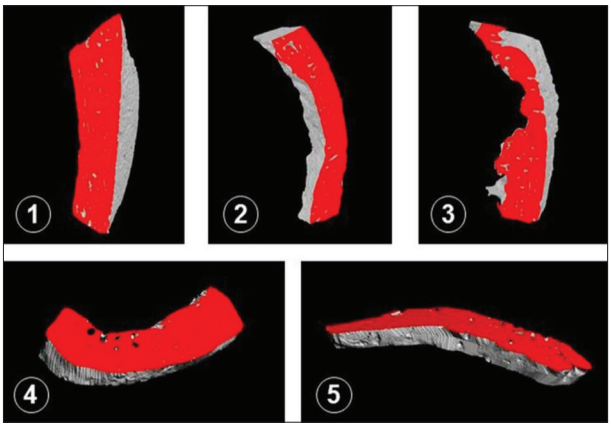

Figure 2. Micro-computed tomography scans 3D reconstruction: 1. supraorbital margin – orbit; 2. glabella; 3. mastoid process; 4. body of the zygoma; 5. pyriform aperture – perinasal

A low-speed diamond saw (SYJ-160; MTI Corporation, Richmond, CA, USA) was used to excise bone specimens from the five sites of the skull that correspond to the common implant placement sites in patients (Figure 1).

The specimens were scanned at the Department of Radiology, School of Dental Medicine, University of Belgrade.

The scanning was performed in a bone window with a voltage of 120 kV and a tube current of 40 mAs. A total of 179 axial sections were obtained with a single slice thickness of 0.75 mm.

Each bone sample was scanned in dry state at a resolution of 10 μm using micro-CT (SkyScan 1172 x-Ray Microtomography; SkyScan, Kontich, Belgium). Acquisitions were performed on 85 kV voltages, 118 μA pipe current, 1000 ms time exposure, 0.5 mm thick aluminum and copper filter, and 180° rotation. The obtained images were reconstructed using NRecon v.1.6.9.8 software (Micro Photonics Inc., Allentown, PA, USA) with a beam hardening correction of 25%, a ring artefact with a correction of 18%, and a reduction of 2. The images were then analyzed using CTAn 1.14.4.1 software (Bruker-microCT N.V. Company, Kontich, Belgium). 3D reconstructions were made (Figure 2).

The following microarchitectural parameters were evaluated: cortical thickness, cortical porosity, pore diameter, and pore separation (Table 1).

Table 1. Microarchitectural parameters of the cortical bone measured by microcomputed tomography

Microcomputed tomography parameter	Unit	Description
Cortical thickness	mm	Average thickness of the cortical bone
Cortical porosity	%	Volume of pores in relation to the total volume of the cortical bone
Pore diameter	mm	Average pore diameter
Pore separation	mm	Average distance between pores

RESULTS

The obtained results were based on micro-CT scanning evaluation of the microarchitectural parameters in five different positions (Figure 1).

The smallest cortical porosity (Ct.Po.; 4.1%) was determined in the glabella, which suggests that this region has the densest cortical bone. The maximum pore separation (Po.Sp.; 0.5 mm) and small pore diameter (Po.Dm.; 0.1 mm) also speak in favor of dense glabellar cortical bone. Moreover, glabellar cortical thickness (Ct.Th.) showed a value of 1.5 mm. The maximum Ct.Th. (2.7 mm) was found in the zygomatic region, as well as the maximum pore diameter (Po.Dm 0.2 mm). In the orbital region, the value of cortical thickness was also high (Ct.Th. 1.9 mm), although the porosity was somewhat higher (Ct.Po. 6.7 %), which tells about thick but porous cortex. The mastoid part of the temporal bone showed the minimum thickness of the cortical bone (Ct.Th. of 1.2 mm), as well as the smallest Po.Sp. (0.3 mm). Perinasal region showed the highest Ct.Po. values (8.5%) (Table 2).

Table 2. Microarchitectural parameters of the cortical bone microcomputed tomography evaluation

Parameter	Position 1 Orbit (supraorbital margin)	Position 2 Glabella	Position 3 Mastoid. Pr.	Position 4 Zygoma.	Position 5 Perinasal (pyriform aperture)
Cortical thickness (mm)	1.9	1.5	1.2′	2.7*	1.4
Cortical porosity (%)	6.7	4.1′	4.3	5.7	8.5*
Pore diameter (mm)	0.1	0.1	0.1	0.2*	0.1
Pore separation (mm)	0.4	0.5*	0.3′	0.4	0.4

*Highest value for the parameter;
′lowest value for the parameter

DISCUSSION

Bone tissue exhibits organization from smaller (nano, micro) to larger (macro) length scales. However, there is a shortage of qualitative information on cortical bone thickness, porosity, as well as on the distribution and size of pore in the midface region and cranium. Therefore, the essence of the present research was to investigate how cortical bone variates in micro-architectural parameters in areas of interest for craniofacial implant placement [5, 6]. Extra-oral (EO) implants are used for anchoring maxillofacial epithesis. A reliable and clinically verified implant therapy includes production of freestanding implant-supported prosthesis [7]. Generally, EO screw-type implants are widely used for this purpose. Due to the anatomical features and thickness of the bone available, the use of conventional EO screw-shaped implants is limited. Good anchoring of enosseal implants requires sufficient bone volume and density [1, 8]. In the case of bone resection, only a small amount of cortical bone is usually left behind. Hence, particularly in the midface area, anchorage of screw-type implants is compromised. The usual locations for screw implant placement are the glabella, mastoid part of temporal bone and upper ridge of orbit. Vertical or even horizontal bone dimensions are often limited after surgery, for example nasal amputation, thus screw type implants often cannot be used [1, 3]. However, disk implants present an optimal alternative whenever implant-retained craniofacial epithesis are indicated, especially when "vertical" bone substance is limited, because such implants require the width rather than the height of bone. Since the thickness of the disk implant plate is 0.6 mm, the minimum amount of the cortical bone where the disk implant could be placed is at least 1 mm, which is far less than minimal requirements for EO screw implants [9, 10]. Disk implants are bi- or multicortically anchored to the cortical bone. The basic premise is that these implants should have absolute primary stability in cortical bone on each side of the disk-plate. The functional load is transferred to the cortical/basal part of the bone [9, 10].

One of the most important factors in the implant therapy is the bone tissue quality. The bone tissue was evaluated and categorized over the years, by different authors [11–15]. However, not a single classification was directly correlated to the implant therapy success. It is not possible to predict the subtle differences in bone quality when applying either the Lekholm and Zarb or Misch classifications [11, 12]. For this reason, Trisi and Rao [13] and Norton and Gamble [14] demonstrated that subjective methods of evaluating bone quality are useful only when clinically assessing up to three classes of bone quality [15].

In spite of this, the use of computed tomography / cone beam computed tomography methods to estimate the degree of bone density is not implemented by implantologists very often.

Nevertheless, the microarchitecture of the bone has an impact on the success of the implant therapy. Microarchitectural parameters like Co.Th., Po.Dm., Co.Po., and Po.Sp. can tell a lot about the bone characteristics, and help predict the outcome of the EO implant therapy in a certain region of the cranium [3, 16].

Micro-CT evaluation can provide an insight to biomechanical properties of the midfacial bones, their thick cortical bone structure, zones of strength, as well as the areas containing thin cortical bone which are considered weak and fragile. However, recent studies revealed that bones of the midfacial skeleton exhibit remarkable regional variations in structure and elastic properties. These variations have been frequently suggested to result from different involvement of cortical and trabecular bone in the transfer of forces. This is why precise evaluation of the areas intended for implantation is important [17, 18, 19].

By examining the microarchitecture of the cortical bone in the orbit, glabella, peripheral region of the aperture piriformis, zygomatic bone, it was understood that the qualitative value of the cortical bone tissue in these localizations was optimal for insertion of disk implants, that are cortically anchored, which is a good alternative for retention of maxillofacial prothesis. This bone area is typically resistant to infection because of its high mineralization. Furthermore, these bone areas are stable to resorption [3, 9, 10]. That is why the cortical bone was of interest for this study.

The maximum Ct.Th. value was in the zygomatic region (2.7 mm) and slightly smaller in the orbital region (1.9 mm). Glabella, piriform aperture (perinasal bone area) showed smaller Ct.Th. (1.5 mm and 1.4 mm, respectively). Because of relatively dense cortical bone in those areas, disk implants can be used [1, 3, 9]. When the microarchitectural parameters were higher (Co.Po. and Co.Th.), and when there is a sufficient amount of bone for triple disk implants, it would be justified to use this kind of implants because of better stability. Single- or double-disk implants could be used in the limited bone quality and quantity when the Ct.Th. is smaller and Co.Po. lower.

Mastoid part of the temporal bone showed the minimum Ct.Th. (1.2 mm) as well as small Ct.Po. (4.3 mm). Anatomically and microarchitecturally, this part of the temporal bone is suitable only for screw EO implants. Screw-type EO implants are similar to short oral (dental) implants; however, there are some differences when it comes to the shape. EO implants have a flange design around their neck to prevent an unwanted drop of the implant, intracranial in the mastoid region. This is justified even more because this region has the smallest cortical thickness, which was shown in this study. For this reason, the implant placement has to be very carefully performed because thin cortex can be easily disrupted [3, 16].

According to other researches where Ct.Th. was higher, the implant stability was more satisfactory. In addition, according to implant stability quotient by resonant frequency analysis, where Ct.Po. was the smallest, Po.Dm./Po.Sp. the greatest, the implant stability was the best. This suggests that the cortical bone characteristics and microarchitectural parameters may determine the outcome of the implant therapy [20–24].

Micro-CT evaluation of cortical bone on the dry scull cadaver model, on certain implant placement points, can give insight into the cortical bone properties, which can

provide valuable guidelines when planning complex implant-retained prosthetic restoration.

CONCLUSION

The bone quality measured through microarchitectural parameters was good in all the regions of interest for the disk- and screw-shape EO implant anchorage.

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Процена кортикалне кости микрокомпјутерском томографијом за краниофацијалну имплантологију

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САЖЕТАК

Увод Добра стабилност имплантата један је од најважнијих фактора за успешну имплантолошку терапију. Овај предуслов је применљив на све типове имплантата, оралне и екстраоралне, краниофацијалне имплантате. Један од најважнијих фактора за задовољавајућу стабилност имплантата је квалитет кости, нарочито кортикалне кости, што је одређено микроархитектонским параметрима.

Циљ је био процена микроархитектонских параметара кортикалне кости на циљаним регијама за постављање краниофацијалних имплантата.

Методе Квалитет кости на циљаним локализацијама одређен је микрокомпјутерском томографијом на кадаверичном моделу. Регије од интереса за постављање имплантата биле су: периорбитална, периназална и периаурикуларна регија. Испитани микроархитектонски параметри су кортикална дебљина (*Ct.Th.*), кортикална порозност (*Ct.Po.*), пречник пора (*Po.Dm.*) и сепарација пора (*Po.Sp.*).

Резултати Најмања *Ct.Po* (4,1%) и највећа *Po.Sp.* (0,5 *mm*) утврђене су у глабели. Највећа *Ct.Th.* (2,7 *mm*) и *Po.Dm.* (0,2 *mm*) пронађене су у зигоматичкој регији. Мастоидни део темпоралне кости показао је најмању *Ct.Th.* (1,2 *mm*) и *Po.Sp.* (0,3 *mm*). Највећа *Ct.Po.* (8,5%) била је у периназалној регији.

Закључак Квалитет кости измерен микроархитектонским параметрима био је задовољавајући у свим регијама од интереса за сидрење екстраоралних имплантата облика диска и шрафа.

Кључне речи: микроархитектура; квалитет кости; микрокомпјутерска томографија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of salivary stress biomarker changes after the insertion of complete dentures

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SUMMARY

Introduction/Objective The purpose of this study was to assess the relationships between salivary stress markers [salivary α-amylase (sAA) and pH], and patients' stress with new dentures.

Methods An intra-individual evaluation was done, in which 30 participants older than 65 years, both sexes, in good general health, first-time complete dentures (CDs) wearers, took part. Measuring of stress biomarkers was done in the unstimulated saliva collected by the so-called spitting method: (1) before any prosthetic treatment, (2) after insertion of a new pair of CDs, and (3) two months after CDs delivery.

Results Upon evaluating the obtained data it was observed that the sAA values steadily increased and by the end of the observation period sAA was higher in women but without statistical significance. The pH values increased until the second follow-up period, after which they dropped. The post-hoc test showed a statistically significant difference in pH values between the first *vs*. the second ($p = 0.005$) and the second *vs*. the third ($p = 0.001$) follow-up periods.

Conclusion There was a non-significant increase in sAA values during the adaptation period. The biggest difference in sAA values between men and women was at the moment of insertion of dentures. The highest pH level was after the insertion of dentures. Since sAA changes were of no statistical significance, the sAA and pH values cannot be used as a reliable biomarker in saliva for observing the patients' adaptation, psychological, and emotional issues.

Keywords: salivary stress markers; complete dentures; elderly

INTRODUCTION

Implant supported restorations are contemporary treatment options in therapy of complete edentulism. Nevertheless, complete dentures (CDs) are still an alternative teeth replacement option in the elderly, especially in developing countries, mainly due to socio-economic reasons. Hence, with optimal retention and stability, conventional CDs may restore oral functions [1, 2].

CDs have proven to be beneficial to patients, improving their masticatory efficiency, esthetics, and socio-psychological well-being. Indisputably, conventional CDs are mucosa-borne appliances, thus exerting the occlusal stress on supporting tissues. Also, it is a rigid appliance, introduced in a very dynamic oral environment, and its functionality depends on the patient's ability to coordinate it together with the activity of the tongue and the masticatory muscles, especially during the unavoidable period of neuromuscular adaptation. Therefore, many requirements are placed before an edentulous patient in order to overcome the limitations of an appliance such as a rigid CD. Hence, beside the findings that prosthesis retention contributes dramatically to prosthesis acceptance by the patient [3], it may be speculated that successful CDs treatment depends on whether or not it presents a stress to a patient's organism.

Stress may relate to usual transitory problems that arise after the insertion of new CDs, such as discomfort, functional difficulties, sore spots, injured mucosa, and different levels of pain, thus extending the period of adaptation [4]. Introducing new CDs may also be a predisposing factor for the onset of salivary changes that affect oral homeostasis and oral mucosal health [4].

Recently, it was summarized that salivary α-amylase (sAA) might be regarded as an indirect indicator of autonomic activation expected during psychological stresses [5]. Everyday stress contributes to sAA changes, whereas the sAA values increase in response to expectations of medical procedures [6, 7, 8]. In a further investigation it was reported that sAA measurement might be a promising approach for studies of treatment effects, as well as a useful marker in the context of pain validation or sleep quality [5, 9, 10]. Together with the most frequently used salivary biomarkers such as cortisol, IgA, and sAA for indicating psychologic stress, pH level was recently introduced as a possible useful and non-expensive biomarker [11, 12].

We hypothesized that the insertion of new CDs may present an overall stressful environment for patients receiving CDs for the first time. Particularly associated with sensory and motor deterioration in older people, some inevitable level of stress may be expected during the first period of coping with new CDs. To our

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knowledge, there are no existing studies dealing with the impact of new CDs on changes in salivary biomarker levels in first-time CDs wearer participants. Therefore, the aim of the study was to evaluate the changes in the levels of sAA and pH of the unstimulated saliva in the elderly who received their first pair of complete dentures during the neuromuscular adaptation period.

METHODS

Study population

The participants for the study were recruited from edentulous subjects seeking prosthodontic treatment and based on matched inclusion criteria.

The inclusion criteria for the study group (SG) were as follows: 1) subjects over 65 years of age, both sexes; 2) healthy, without oral and systemic diseases that may influence the saliva quality and quantity; 3) first-time wearers of CDs without previous experience in wearing mobile appliances.

Individuals with previous experience in wearing dentures, persons with motor or neurological disorders, and smokers were excluded from the study. In addition, the exclusion criterion included using medications that may affect the sympathetic or parasympathetic nervous system.

A total of 30 participants (13 male and 17 female) were allocated to the SG. The participants were instructed not to eat or drink within two hours before saliva collection and all were informed on the study procedures and all provided their written consent.

Study design

The study plan included an evaluation of salivary stress biomarker (sAA and pH) values in the unstimulated saliva collected by the so-called spitting method [13].

SG participants were instructed to sit comfortably, with eyes open, and rinse their mouths for five seconds with 5 ml of distilled water. Afterwards, they were asked to collect their saliva by spitting into a plastic tube every 30 seconds until 5 ml of saliva were collected.

Sampling procedure in the SG was performed in three investigation steps according to the investigation protocol:

1. Sampling and saliva testing before any prosthetic treatment;
2. Sampling and saliva testing after the insertion of a new pair of CDs (saliva sampling was performed during the first half of an hour, when the CDs were placed and re-occluded);
3. Sampling and saliva testing two months after the CDs delivery, assuming that the initial neuromuscular adaptation period was finished.

The saliva was taken for all three investigation steps in the SG under the same clinical conditions, between 1 pm and 2 pm. The study design and data collection methods were approved by the Ethics Committee of School of Dental Medicine (No. 36/26), and were performed in accordance with the Declaration of Helsinki.

Determination of salivary biomarkers in saliva samples

After the sampling, the saliva was not frozen and the markers in it were analyzed within an hour. All the samples were centrifuged at 3500 rpm for 15 minutes. During the analysis phase, pH values in saliva were measured, using pH meter (Martini Instruments, USA), calibrated with standard solutions with a pH of 4 and 7, respectively. The sAA values were determined using the colorimetric method and a commercial kit (Alpha-Amylase LiquiColor, Human, Wiesbaden, Germany) following the International Federation of Clinical Chemistry and Laboratory Medicine method [14]. The sAA activity of the samples was detected through the enzymatic hydrolysis of the chromogenic substrate 2-chloro-4-nitrophenyl-D-maltotrioside to 2-chloro-nitrophenol (CNP). The rate of increase in absorbance, due to the formation of CNP, is measured at 405 nm and is proportional to the sAA activity in the sample. Amylase activity was expressed in units per milliliter (U/ml).

Saliva samples were analyzed at the Laboratory for Biochemistry and Haematology, School of Dental Medicine, University of Belgrade.

Statistical analysis

The data was analyzed in IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics for numeric data were presented by the mean and standard error. The numeric data were analyzed using the Mann–Whitney test or the t-test. One-way ANOVA with repeated measures was used for the analysis between the biomarkers of saliva at three periods (before, after handing over the dentures, and after two months). Mixed between-within subject ANOVA was used to evaluate the effect of two factors (time and sex) on the biomarkers. Spearman's correlation coefficient was done in order to assess the relationship between the saliva biomarkers and clinical parameters. A p-value less than 0.05 was considered statistically significant.

RESULTS

The values of sAA and pH for all patients (male and female) are presented in Table 1. Statistically significant differences were observed between the pH values during the study. A subsequent post-hoc test showed a statistically significant difference in pH values between the first and the second follow-up period (before *vs.* after handing over the dentures, $p = 0.005$) and between the second and the third period (after handing over dentures *vs.* after two months, $p = 0.001$). The sAA values increased during the evaluation period, but this was not statistically significant (Table 1).

The sAA values were slightly higher in women than in men, but with no statistically significant difference. The pH values were similar between the sexes and there were no statistically significant differences during the observation period (Table 2).

The mixed-design ANOVA results show a significant time influence on the pH values ($p = 0.002$), while the influence

Table 1. Monitoring of stress factors (salivary α-amylase and pH) in all participants during all study periods

Biochemical parameters Mean (SE)	Evaluation period			p	
	Before	After handing over dentures	After 2 months		
Salivary α-amylase	264.0 (49.9)	290.0 (50.9)	318.0 (44.3)	0.680[a]	
pH	7.3 (0.5)	7.7 (0.1)	7.1 (0.1)	0.001*[a]	1 *vs.* 2, p = 0.005*[b] 2 *vs.* 3, p = 0.001* 1 *vs.* 3, p = 0.119

[a]One-way ANOVA with repeated measures;
[b]post-hoc analysis: Bonferroni test;
*statistically significant

Table 2. Comparison values of salivary α-amylase and pH between sexes during the study period

Variables /Mean (SE)	Male	Female	p
Age	66 (1.3)	65 (0.9)	0.837
Salivary α-amylase			
Before	269 (66.7)	241.3 (54.5)	0.752
After handing over dentures	295.2 (62.6)	252.7 (66.1)	0.652
After 2 months	300.3 (54.1)	332 (68.4)	0.728
pH			
Before	7.2 (0.2)	7.4 (0.1)	0.382
After handing over dentures	7.6 (0.2)	7.8 (0.1)	0.327
After 2 months	7.1 (0.1)	7.1 (0.1)	0.686

of sex and time combined is of no statistical significance. Changes in pH values were similar for both men and women: initially we have an increase, followed by a decrease in values after the second follow-up period. The pH values are higher in men (Figure 1). The influence of time (p = 0.703) and time and sex (p = 0.679) on the sAA values was not statistically significant, but initially there were lower sAA values in women, which increased during the observation period, while in men there were higher sAA values which reduced over time. The largest difference in sAA values between the sexes was immediately after the dentures were handed over (Figure 2).

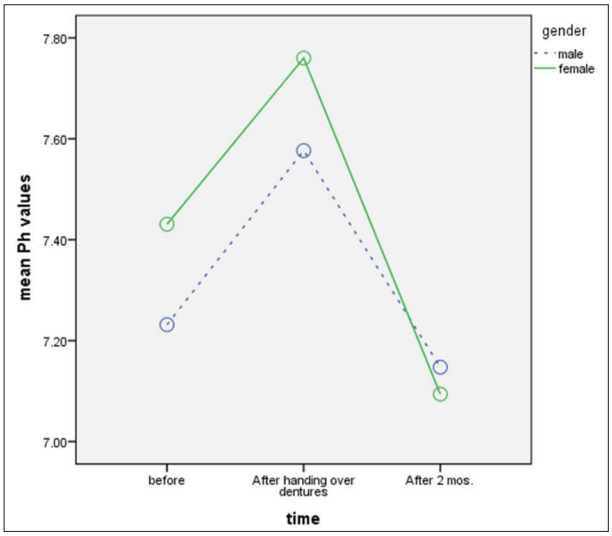

Figure 1. Effects from two factors (time and sex) on pH levels

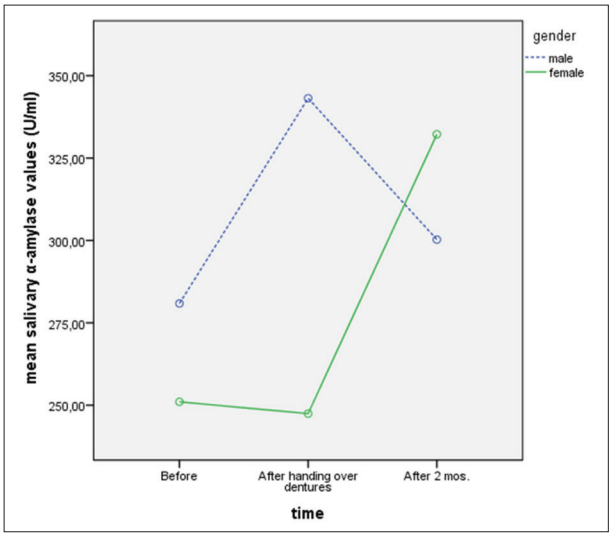

Figure 2. Effects of two factors (time and sex) on salivary α-amylase levels

DISSCUSION

First-time CDs wearers are under various factors that influence their adaptation period. These factors are not fully understood and are addressed as follows: the quality of dentures, oral conditions, patient–dentist relationship, attitude toward dentures, patient's personality, and socio-economic factors [4]. Clinical problems are mostly easily recognized, but the psychological discomfort during the adaptation period has not been fully explained. This follow-up study assessed the changes of salivary biomarkers, sAA and pH values, during the period of neuromuscular adaptation in patients who received their CDs for the first time.

Most of the saliva research focused on the saliva's role in digestion, as a lubricant for food and tongue, and in microbiological balance of the oral cavity [15, 16, 17]. Furthermore, biomarkers from saliva are reliable, non-invasive, and objective, and may be used for monitoring oral health or therapy outcomes [18, 19]. The relevance of saliva for the success of denture retention and oral comfort has been underlined; thus, the knowledge of the salivary biomarkers further emphasizes the factors that contribute to CDs being utilized more comfortably and easily [20].

During the adaptation period and the process of learning to use dentures, patients consciously and unconsciously promote more chewing strokes. Thus, when more mastication was needed, significant changes have been observed in the salivary flow rate with consequent changes in amylase and protein concentration, as well as in an increase of pH values [21]. In addition to that, a study reported a statistical significance of pH values before and after the insertion of CDs [22]. Others, however, have not found significant changes in pH values [4, 23]. Findings of our study are in correlation with previous findings that pH is significantly different before and after dentures' insertion [24]. The differences in the reported pH values can also be explained

by the different methods used for the measurements (pH-measuring tapes or electronically).

All the patients included in the SG were first-time denture wearers and it was expected that they would feel stress related to the initial foreign body sensation. However, the findings of the study did not support this hypothesis. Although the sAA and pH values changed during the observation period, they were not of statistical significance, especially in regard to sAA values. Studies show that the sAA values increase when an individual undergoes stress. The sAA value increases within three minutes of watching a stressful video, or five minutes into a mental arithmetic task. In these situations, the sAA values increase 100% and 200%, respectively, while these values lower within three minutes of undergoing soothing conditions. Thus, we can explain that dentures do not present great psychological stress for the patient who is exposed to effects of other factors, during such a long observation period that may have a greater impact [25]. The sAA values higher in men than in women are shown in the research by Matsui et al. [26]. They determined that older men react to denture wearing with a significant increase in sAA values. If we look at the whole adaptation period of two months, we have a steady increase of sAA values in women. Rafeeq [27] arrived at a similar conclusion; however, in his research, the cortisol level, as a stress biomarker, was higher in women during the period of adaptation to new dentures, indicating that women are more susceptible to stress.

In our study, there is a period of adaptation of the oral cavity to the new dentures, which probably affects the composition of the saliva. Therefore, the sense associated with new dentures during the whole adaptation period of two months may increase the sAA level in saliva, but not enough to be of statistical significance. This finding might indicate an autonomic response of the body as it adjusts to wearing new dentures, because our mind is receiving strong signals from the mouth that new dentures are present.

When pH values were analyzed, a significant increase was observed in the SG during the observation period, but the opinion is that an increase in pH values was due to the enhancement of salivary flow rate accompanied by the insertion of the initial set of CDs [24–29]. Some researchers speculated that CDs in the mouth acted as foreign bodies that simulate saliva flow [30]. However, it is more likely that continuous pressure of CD's prolonged the stimulation of mucosa mechanoreceptors, that initiates enhanced salivary flow [25, 31]. We are more prone to believe that such an enhancement of salivary flow, rather than psychological stress that CDs patients are exposed to, influences the salivary composition and the increase of investigated sAA and pH values.

According to the psychological models of stress, threat and challenge appraisals have a major effect on the degree of stress experienced by individuals and their emotional reactions to a stressful situation [11]. However, the study was initiated by the idea that patients were exposed to worries about how well they would cope with new CDs. Whereas worries are more related to appraisals, which constitute more of a challenge than a threat, activating the nervous system in a different way [11], worry dimension rather than emotional dimension is associated with various physiological markers, such as cardiovascular responses [32]. However, threat appraisals, not challenge ones, are more associated with pH as stress markers [11]. Accepting the aforementioned and summarizing the obtained study findings, it is more likely that new CDs present a challenge, not a threat to the organism, as it was not evident, according to stress level markers, that inserting new CDs induced a stress reaction.

In order to analyze only the impact of CDs on saliva stress biomarkers, two main health issues were excluded prior to the sampling, as they might have influenced the objectivity of the results. Participants of both groups were non-smoking individuals, due to a reported statement that smoking increases the stress hormone levels, with associated changes in pH [33]. Also, the participants were instructed to sit calmly, restraining from any physical activity prior to the sampling, since there is a firm association between physical activity and stress levels.

Although designed as a pilot study, the main limitation is the relatively small number of participants with a reflective lack of a possible generalization of the findings. We did our best to keep the SG as homogenous as possible; the dentures were constructed by the same dentist and dental technician, using materials from the same manufacturer with satisfactory retention and stabilization. Moreover, since it is well known that sAA and salivary pH values change throughout the day, variance of daily values and subsequent influence on the findings may be another limitation.

CONCLUSION

The sAA values are increased during the adaptation period to new CDs. The changes in sAA values in regard to the participant's sex show that sAA values in women increase over time, while the sAA values in men decrease. The study shows that the highest pH value occurs immediately after the dentures are delivered. Since sAA changes are of no statistical significance, sAA and pH values cannot be used as reliable biomarkers in saliva for monitoring the patient's adaptation, psychological, and emotional issues.

Conflict of interest: None declared.

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Анализа маркера стреса у пљувачки код пацијената који први пут носе тоталне протезе

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САЖЕТАК

Увод/Циљ Циљ ове студије је био да се изврши анализа маркера стреса у пљувачки α-амилазе (сАА) и *pH* код пацијената који први пут добијају протезе.

Методе Извршена је интраиндивидуална анализа у којој је учествовало 30 болесника старијих од 65 година, оба пола, доброг општег здравственог стања који су први пут добили тоталне протезе. Мерење маркера стреса извршено је у нестимулисаној пљувачки прикупљеној методом тзв. пљувања и то: (1) пре било какве протетске терапије, (2) после предаје новог пара тоталних протеза и (3) два месеца после предаје тоталних протеза.

Резултати Анализом добијених резултата уочава се стални раст вредности сАА, и на крају опсервационог периода сАА је већи код жена, али без статистичке значајности. Вредности *pH* се повећавају до другог периода праћења и тада опадају. *Post-hoc* тест је показао статистички значајну разлику у вредностима *pH* између првог и другог ($p = 0,005$) и другог и трећег ($p = 0,001$) периода праћења.

Закључак Током периода адаптације забележен је незнатан пораст вредности сАА. Највећа разлика у вредности сАА између мушкараца и жена је забележен у тренутку предаје нових протеза. Будући да промене сАА немају статистички значај, вредности сАА и *pH* се не могу користити као поуздан биомаркер у пљувачки за посматрање болесниковог прилагођавања и постојање психолошких и емоционалних проблема.

Кључне речи: маркери стреса у пљувачки; тотална протеза; старије особе

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Foramen of Vesalius – constant or variable foramen

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SUMMARY

Introduction/Objective The foramen of Vesalius is a variable foramen located at the base of the skull, anteromedial to the foramen ovale, and lateral to the foramen rotundum. Through this foramen, passes one of the emissary veins, which establishes communication between the cavernous sinus and the pterygoid plexus.

The aim of the study was to determine the incidence of this foramen in adults depending on gender, along with the number of foramina, distributions relative to the side of the skull and diameter of the foramen.

Methods A material used in the study were digital computed tomography (CT) scans of adult paranasal cavities from the archives of the Radiology Center, archived in the PACS software system. We analyzed axial CT sections of 1 mm thickness. The research included 500 subjects (250 males and 250 females).

Results The foramen of Vesalius was present in 67.6% of respondents. In 50.9% cases, the foramen was bilateral and in 49.1% it was unilateral. The average oblique diameter of the foramen in men was 1.75 ± 0.59 mm and in women 1.56 ± 0.48 mm. In 22 subjects (6.51%) the foramina were doubled, and in two (0.60%) they were tripled.

Conclusion There was no statistically significant difference in the incidence of the foramen of Vesalius concerning gender. The mean diameter of the foramen was statistically higher in males. The presence of this foramen is important for neurosurgeons because, during the percutaneous trigeminal rhizotomy, the needle can pass through this foramen, injure the surrounding blood vessels, and lead to intracranial hemorrhage.

Keywords: foramen of Vesalius; CT scans; subjects; mean diameter

INTRODUCTION

There are many foramina on the floor of the middle cranial fossa, which are important because they allow the passage of essential structures such as nerves and blood vessels. The permanent apertures of the sphenoid bone are foramen rotundum, foramen ovale, and foramen spinosum, whereas the foramen of Vesalius (FV) and meningoorbital (Hyrtl's channel) foramen represent non-permanent foramina [1].

The FV is a small, variable foramen located anteromedial to foramen ovale and posterolateral to foramen rotundum in the middle cranial fossa [2]. The FV (sphenoid emissary foramen) was first described and drawn by the anatomist Andreas Vesalius, after whom the foramen was named. The FV can be unilateral or bilateral. The mean diameter of this foramen in the adult is 1.4–2 mm [3].

The FV transmits one of the emissary veins that connect the cavernous sinus and the pterygoid plexus [4]. Emissary veins play a role in maintaining the balance between intracranial and extracranial pressure. Under physiological conditions, blood flow through the emissary veins is small, but in conditions of increased intracranial pressure, these veins become an important blood drainage pathway [5]. According to Lang [6], a small nerve (lateral sphenoid nerve) can pass through the FV on the way to the cavernous sinus.

According to Wood-Jones [7], the FV is an indicator of the evolutionary complexation of the cranial venous system of man. The FV does not exist in any primate other than humans.

Anthropological research has shown a significant discrepancy regarding the prevalence of the FV between various authors [8]. Because of this, we have analyzed the digital data of computed tomography (CT) images of adult human skulls to determine the representation of the FV in the human population.

The aim of the study was to determine the incidence of FV in adults. In addition, the goal was to determine the number of foramina, the diameter of the foramina, and the foramen distribution related to the side of the skull.

METHODS

In this retrospective study, we used digital CT scans from adults that were archived in the Picture Archiving and Communications System

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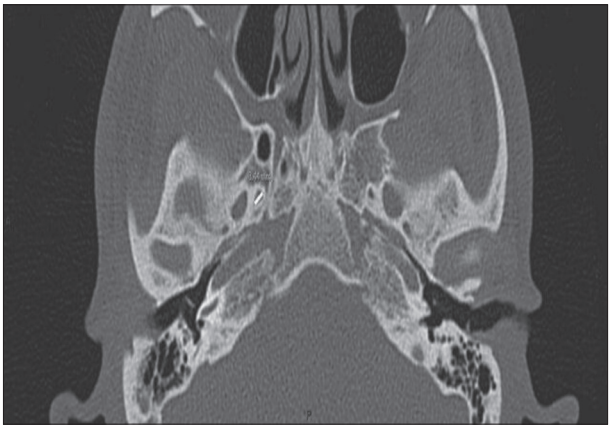

Figure 1. Measurement of the oblique diameter of the foramen in the PACS software (the white line represents foramen of Vesalius diameter)

(PACS) software system of the Radiology Center of the Clinical Center of Vojvodina. The patient's recordings were made as part of a CT scan of the paranasal cavities on the CT scanner Siemens Sensation 64 (Siemens AG, Siemens Healthineers AG, Erlangen, Germany). The study included 500 subjects, of which 250 were male and 250 were female. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad.

We analyzed axial CT sections of 1 mm thickness and resolution H60s. The foramen was measured using a digital measuring tool of the PACS software. The largest oblique diameter of the foramen at the axial section was measured (Figure 1).

The images were evaluated for the presence or absence and unilateral or bilateral pattern of FV. Including criteria were localization of the foramen – anteromedial to foramen ovale and posterolateral to foramen rotundum, visibility on both surfaces of the sphenoid bone, and a maximum oblique diameter greater than 1 mm. The cut off value of 1 mm regarding maximum oblique diameter was used to avoid misidentification with a spongy bone of the skull base. Obtained results were presented in figures and tables.

The software program SPSS v.21.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) was used for storing and analyzing data. The results of numerical continuous variables were presented as arithmetic mean and standard deviation. The categorical variables were compared by the χ^2 test and the independent samples t-test. The significance level value was set at 0.05.

RESULTS

In the sample of 500 subjects, we analyzed the presence of the FV. The foramen was present in 338 subjects (67.6%) and absent in 162 (32.4%) subjects (Figure 2).

In our sample (Table 1), the FV was observed in 165 (66%) male and 173 (69%) female subjects. During data processing, it was found that there was no statistically significant difference in the FV incidence between male and female skulls ($\chi^2 = 0.584$, $p = 0.444$).

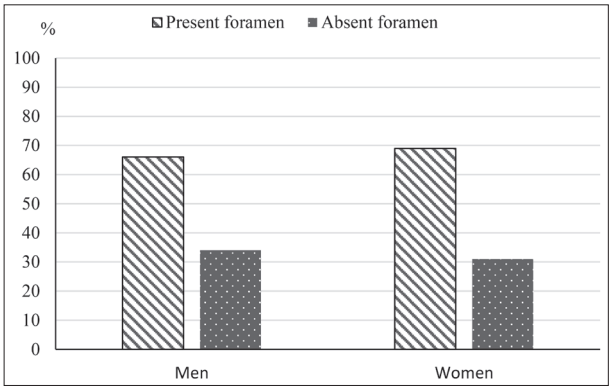

Figure 2. Percentage representation of the foramen of Vesalius

Table 1. Frequency of occurrence of the foramen of Vesalius concerning the sex

Gender	Present foramen	Absent foramen	Total
Men	165 (66%)	85 (34%)	250 (100%)
Women	173 (69%)	77 (31%)	250 (100%)

Table 2. Distribution of the presence of foramina concerning the sex

Sex	Unilateral foramen	Bilateral foramen	Total
Men	71 (43%)	94 (57%)	165
Women	96 (55.5%)	77 (45.5%)	173
Total	166 (49.1%)	172 (50.9%)	338

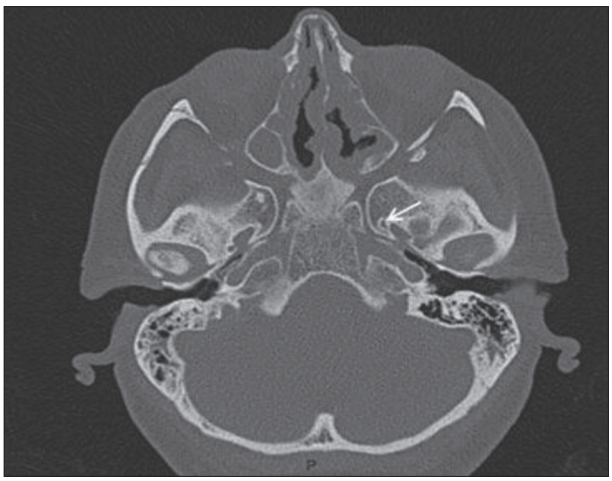

Figure 3. Unilateral foramen (white arrow) on a computed tomography scan

The FV was observed bilaterally in 50.9% cases. The incidence of unilateral foramen in this study was 49.1% of which 46.6% on the right side and 53.4% on the left side of the skull. (Figure 4).

Examining 165 male skulls, a bilateral foramen was present in 57% of cases and unilateral in 43%. In 173 female subjects the FV was bilateral in 77 (45.5%) skulls and in 96 (55.5%) unilateral (Table 2). Bilateral foramen was more common in men and unilateral in women ($\chi^2 = 4.759$, $p = 0.0291$).

In 124 male subjects (47.1%), the foramen was located on the right side and in 139 (52.9%) on the left side of the skull (Figure 5). There was no statistically significant difference ($\chi^2 = 0.0147$, $p = 0.903$) considering the

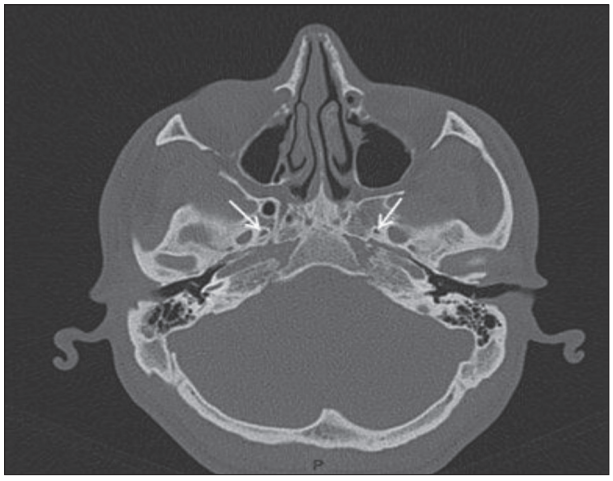

Figure 4. Bilateral foramen (white arrow) on a computed tomography scan

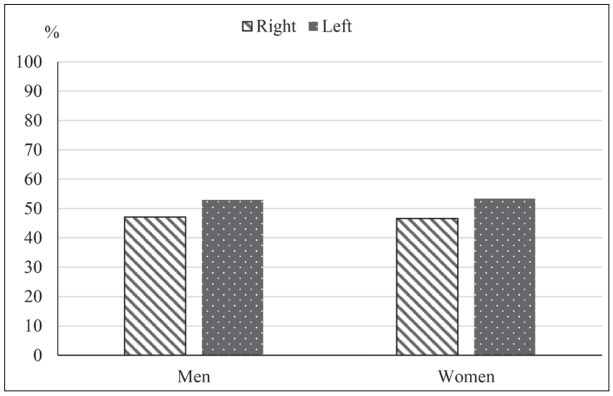

Figure 5. Distribution of foramina concerning the side of the skull and the sex

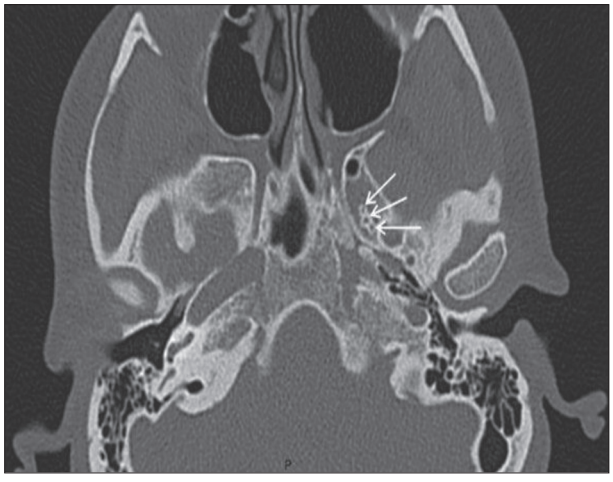

Figure 6. Triple foramen of Vesalius (white arrows) on computed tomography scan

distribution of the FV between the sexes in relation to the side of the skull.

Considering the measurements of the largest oblique diameter of the FV, all foramina were classified into seven groups (Table 3). A total of 226 foramina were 1–1.49 mm in diameter, which is the most commonly recorded foramen size. The largest diameter of the foramen was 4.48 mm and was recorded in one male subject.

Table 3. The maximum oblique diameter of the foramen in millimeters (arranged in groups)

Diameter of the foramen (mm)	Men	Women	Total
1–1.49	100	126	226
1.5–1.99	64	77	141
2–2.49	45	22	67
2.5–2.99	22	5	27
3–3.99	6	2	8
3.5–3.99	3	2	5
4–4.59	1	/	1

Table 4. Percentage of the foramen of Vesalius in different races and peoples

Author and year	Country	Sample number	Percentrage of the foramen of Vesalius
Boyd (1930)	England	1500 skulls	36.5%
Lanzieri (1988)	America	50 CT scans	64%
Ginsberg (1994)	America	123 CT scans	80%
Kodama (1997)	Japan	400 skulls	21.75%
Gupta (2005)	India	35 skulls	42.90%
Ramalho (2007)	Brazil	80 skulls	71.87%
Kaplan (2007)	Turkey	10 skulls	100%
Shaik (2007)	India	125 skulls	36%
Shinohara (2010)	Brazil	400 skulls	33.5%
Nirmala (2014)	India	180 skulls	50%
Raval (2015)	India	150 skulls	60%
Murlimanju (2015)	India	78 skulls	37.2%
Bayrak (2018)	Turkey	317 CBCT scans	28.1%
Costa do Nascimento (2018)	Brazil	194 skulls	18.55%
Nayak (2018)	India	30 skulls	30%
Leonel (2019)	Brazil	170 skulls	45.2%
Kaplan (2019)	Turkey	350 CBCT scans	41.1%
Maletin (2019)	Serbia	26 skulls	61.54%
Görürgöz (2020)	Turkey	269 CBCT scans	73.1%
Our current work (2020)	Serbia	500 CT scans	67.70%

CT – computed tomography; CBCT – cone-beam computed tomography

The average value of the oblique diameter of the FV in the whole sample was 1.66 ± 0.55 mm. The average diameter of the foramen on the right side of the skull was 1.62 ± 0.55 mm and the left one was 1.69 ± 0.54 mm. We found no significant difference in the size of the foramen diameter compared to the lateral distribution ($t = 1.015$, $p = 0.856$).

The average diameter of the foramina in the male subjects was 1.75 ± 0.59 mm, while in the female subjects it was 1.56 ± 0.48 mm. The average diameter of the FV was significantly higher in male than female skulls ($t = 15.65$, $p = 0.000$).

During the research, double and triple foramina were noticed in a certain number of respondents. Double foramina represent two foramina, while triple foramina represent three foramina located on the same side of the skull placed next to each other. The presence of a double foramen was

noted in 22 (4.4%) out of 500 analyzed skulls. Twenty-two subjects (5.92%) had double FV on one side of the skull, while one subject (0.3%) had double foramina on both sides of the skull. Triple foramina were found in two female subjects (0.6%), and it was located on the left side in both skulls (Figure 6).

DISCUSSION

In this study, which included digital data of 500 CT scans of adults, the presence of the FV was detected in 67.7% of cases. These results are similar with those reported by Görürgöz et al. [4] with 73.1% presence, Maletin et al. [9] in which FV was present in 61.54% of dry adult skulls, and with the study of Lanzieri et al. [10], Raval et al. [11], where the FV was present in 64% and 60% of the respondents. In their research, Shinohara et al. [12] and Shaik et al. [13] state that the foramen representation is 33.75% and 36%, respectively, which is less than the results of our study. According to most authors, the incidence of the FV is lower (Table 4) [3, 12–20]. There is a significant discrepancy in the incidence of the FV between various authors. This may be explained by the different number of examined subjects, ethnicity, and differences in FV identification methodology which can be the subject of future research.

In our sample, there is approximately equal representation of bilateral and unilateral foramina. This finding correlates with the results of Shinohara [12], Gupta [20], and Nirmala [21]. Based on our data we can disagree with the results of the previous studies, which reported that the incidence of the bilateral foramen is higher than unilateral foramen in adult skulls [17, 22, 23].

Regarding the incidence of the FV, we observed no remarkable differences in the ratio between the male and the female subjects (Table 1). This result is consistent with the findings of Kodama et al. [22] whose research included 400 adult human skulls. Unlike our results, some authors reported that the foramen is more common in female subjects [24]. In a study by Chaisuksunt et al. [25] the FV is more likely to occur in males than in females, which can be explained by the higher number of male subjects included in the study (246 males and 131 females).

Analyzing data, we found that bilateral FV was significantly more common in males and unilateral in females. Kodama et al. [22] did not find a difference in the distribution of unilateral and bilateral foramina concerning gender, while other studies did not address this issue.

According to Andreas Vezalius's book titled *De humani corporis fabrica librica septem* [2], there is no remarkable difference between the left and right side regarding the lateral distribution of the FV. The results of our study correlate with the citation of the mentioned well-known anatomist, unlike Rossi et al. [24] which pointed out that the FV is more frequent on the right side of the skull, while in some cases it was more common on the left side [18].

Based on the results of our study, the mean diameter of the FV on the right side was 1.62 ± 0.55 mm and on the left side was 1.69 ± 0.54 mm. These data are similar to the results of Kodama et al. which state that the mean foramen diameter on the left is 1.59 ± 0.94 mm, while on the right it is 1.46 ± 1.04 mm [22], as well as in the study of Görürgöz et al. [4] with right side FV diameter 1.75 ± 1.33 mm and 1.75 ± 1.2 mm on the left side. In the results of the study by Shinohara et al. [12] the average foramen size on the right side is 0.67 ± 0.28 mm and on the left side is 0.76 ± 0.39 mm, which is smaller than the results of our study. Unlike the mentioned studies, Bayrak et al. [16] stated that the mean diameter on the right side is 2.66 mm, and 2.82 on the left side, which is bigger than the results of our study. To the best of our knowledge, there are no possible explanations for the difference in the diameter of the FV in different authors in contemporary literature. The discrepancy in the data of our study and other reports regarding the mean diameter of FV may be the result of a different methodology in defining the FV. Due to the potential false-positive findings, foramina with a diameter smaller than 1 mm were excluded from our study.

In male subjects, the average foramen diameter is 1.75 ± 0.59 mm, while in female subjects it is 1.56 ± 0.48 mm ($t = 15.65$, $p = 0.000$). After statistical data processing, it was found that the size of the foramen diameter was larger in men than in women, whereas in the study by Prakash and Viveka [26] the average diameter of the foramen in males is significantly lower than females. Görürgöz et al. [4] did not find a significant difference in the FV diameter between genders.

The clinical significance of the FV lies in the fact that it provides passage to the emissary vein. Through the FV, septic thrombus can reach from extracranial veins to the cavernous sinus causing cavernous sinus thrombosis. Thrombosis of the cavernous sinus is a very serious condition that can be lethal in up to 30% of the cases or cause serious complications such as ophthalmoplegia, blindness, or cerebrovascular insult. The most likely cause of this pathological condition is an infection within the orbit, paranasal sinuses, or tissue of the upper half of the face [27]. In addition, the FV also has surgical significance. One of the treatment options for trigeminal neuralgia is radiofrequency rhizotomy. During this procedure, while approaching the trigeminal nerve, a needle is inserted intracranially through foramen ovale. When reaching the foramen ovale, the needle may erroneously pass through the FV leading to cavernous sinus puncture and intracranial hemorrhage, which could be life-threatening conditions [28, 29].

Since the FV is not as rare as previously thought, the presence of an excess number of foramina should be suspected during the diagnostic examination of the middle cranial fossa [30]. The presence of the FV, its localization, and diameter are important information for anatomists, radiologists, maxillofacial surgeons, and neurosurgeons.

CONCLUSION

The FV is inconsistent foramen of the base of the skull. The incidence of FV (67.7%) in this is study is considerably

higher than in most previous studies, so FV is not that uncommon finding as previously thought. Regarding the gender incidence of the FV, there is no statistically significant difference between males and females. There is no remarkable difference in the lateral distribution of the FV. The mean diameter of the FV is significantly higher in men than women. We found an interesting fact that in addition to one FV, there may be double and triple foramina, which were found in 22 subjects (6.5%) and two subjects (0.6%) respectively. The exact cause of variations observed in the current study is difficult to determine but these might be due to genetic, nutritional, environmental, or other unknown factors.

Detailed knowledge of the anatomy of the base of the skull and its varieties, including the FV, is very important to the maxillofacial surgeons and neurosurgeons for performing successful and safe microsurgical procedures.

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Везалијусов отвор – сталан или несталан отвор

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САЖЕТАК

Увод/Циљ Везалијусов отвор је варијабилан отвор који се налази на бази лобање, испред и унутра од овалног отвора, а споља и иза округлог отвора. Кроз Везалијусов отвор пролази једна од емисарних вена која успоставља комуникацију између кавернозног синуса и криластог венског сплета. Циљ истраживања био је утврђивање учесталости Везалијусовог отвора код одраслих особа у зависности од пола, као и утврђивање броја отвора, дистрибуција у односу на страну лобање и одређивање дијаметра отвора.

Методе Као материјал коришћени су дигитални подаци снимака параназалних шупљина одраслих особа урађених компјутерском томографијом из архивског материјала Центра за радиологију, који су архивирани у софтверском систему *PACS*. Анализирани су аксијални пресеци КТ дебљине 1 *mm*. Истраживање је обухватило 500 испитаника (250 мушког и 250 женског пола).

Резултати Везалијусов отвор је био присутан код 67,6% испитаника. У 50,9% случајева отвор је био билатералан, а у 49,1% случајева унилатералан. Просечни коси дијаметар отвора код мушкараца износи 1,75 ± 0,59 *mm*, а код жена 1,56 ± 0,48 *mm*. Код 22 испитаника (6,51%) уочени су удвојени, а код два испитаника (0,60%) утројени отвори.

Закључак Нема статистички значајне разлике у учесталости јављања Везалијусовог отвора у односу на пол. Просечни измерени дијаметар Везалијусовог отвора је статистички био већи код мушкараца. Присуство Везалијусовог отвора представља важан податак за неурохирурге, јер током перкутане тригеминалне ризотомије игла може да прође кроз овај отвор, повреди околне крвне судове и доведе до интракранијалне хеморагије.

Кључне речи: Везалијусов отвор; снимци комјутеризованом томографијом; испитаници; просечни дијаметар

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Protection of health workers employed in a tertiary health institution from hepatitis B virus infection

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SUMMARY

Introduction/Objective More than 300 million people around the world are infected with hepatitis B virus (HBV). It is transmitted through blood, blood derivatives, sexually, and vertically, and healthcare workers, due to the nature of their work, represent a vulnerable group.

The aim of this research is to determine the coverage of vaccination against HBV infection of health workers working in a tertiary health institution – the Clinical Centre of Vojvodina, the level of protection by determining anti-HBs antibodies, the exposure degree, the degree of examinee's compliance with implemented protection measures in the workplace, and the level of knowledge about post-exposure prophylaxis (PEP) measures.

Methods The research was conducted as a descriptive analytical cross-sectional study, in which a questionnaire on protection of health workers from blood-borne diseases (BBD) was used as an instrument for research, as well as blood sampling to determine HBs antibody titer. The sample covered 100 health care workers.

Results The research showed a large coverage of vaccination against HBV infection (97%). Aside from continuous seroprophylaxis, 7% of examinees did not have protective anti-HBs antibodies. Health workers' level of exposure to HBV infection incidence is 90%. Protection measures in the workplace are applied by 89% of examinees, whereas 86% are familiar with the PEP measures.

Conclusion The research showed a large coverage of health workers using specific HBV infection protection, insufficiently implemented protection, high exposure to HBV infection incidence, incomplete compliance with safety measures and insufficient knowledge of PEP measures.

Keywords: anti-HBs antibodies; health workers; hepatitis B; incident; safety at work

INTRODUCTION

Hepatitis B virus (HBV) infection represents a global public health problem, due to its high rate of prevalence and severe consequences upon the health of the affected [1, 2]. Data by the World Health Organization (WHO) indicate that a third of the world population is infected with the hepatitis B virus, and 10–30 million newly infected people are registered annually, whereas as much as one million people die as a consequence of the infection caused by the virus [3, 4].

Health care workers (HCWs) are under occupational risk from blood-borne diseases (BBD) [5, 6]. The main BBD prevention measure in health institutions is to avoid exposure (professional exposure), apply hepatitis B vaccines, and adequate post-exposure prophylaxis (PEP) [7, 8]. In the Republic of Serbia there is no precise data on the coverage and the degree of HCWs' specific protection from hepatitis B virus, the number, and the type of incidents in the workplace and PEP, even though there are certain legal provisions governing this issue (Rulebook on Immunization and Protection Measures Using Medicines, Law on Protection of Population Against Infectious Diseases, European Guide for Prevention of Blood-Borne Diseases Transmission, Statistical Yearbook of the Republic of Serbia) [1, 9–12].

Infections caused by HBV among HCWs can be prevented by vaccination [13]. However, vaccination efficacy is not absolute and it correlates with achieved immunological response, represented by the level of HBs antibodies. Post-vaccination immunity is established when the level of anti-HBs antibodies is > 10 mIU/ml [14]. There are several factors that influence the level of HBs antibodies: vaccine factors (dose, schedule, the location of vaccine administration, time after vaccination), and host factors (senior age (40+), male sex, obesity, smoking and chronic diseases) [12].

Beyond the level of HBs antibodies, other factors that impact the risk of infection in

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health workers are the type of needle used, i.e. the instrument that caused the injury, the characteristics and the severity of the injury, the type and amount of the potentially infected fluid, i.e. inoculum, the patient's viremia degree [12].

However, the only conclusive evidence that a post-vaccination immunity has been established is the anti-HBs antibodies' control, and yet, in our country, these levels are not monitored after health workers complete the HBV infection immunization procedure.

The aim of this research was to determine the coverage of vaccination against HBV infection, the level of protection against HBV infection by determining anti HBs antibodies, employee exposure, compliance with the work safety measures, and knowledge about the PEP measures.

METHODS

The research was conducted as a descriptive analytical cross-sectional study. The data were collected by surveying examinees with a questionnaire, which they filled out themselves, and by blood sampling in order to determine anti-HBs antibodies (in February and March of 2019).

The research included 100 health workers employed in a tertiary health institution in the Republic of Serbia – the Clinical Centre of Vojvodina in Novi Sad, in the organizational units in which employees very often come into contact with patients' biological material, i.e. where employees are more exposed due to the nature of services provided to patients (Infectious Diseases Clinic, Emergency Center, Centre for Laboratory Medicine, and Dialysis Unit).

Along with survey questionnaires, the examinees received a designated data sheet with the basic information about the research. The examinees were required to sign an informed consent.

The questionnaire on BBD protection of health workers was used as the research tool designed specifically for this purpose, based on the literature data and examiners' experience.

The questionnaire on BBD protection of health workers consisted of 40 questions divided into four parts. The first part related to general questions, regarding socio-demographic data and the workplace itself, and contained 10 questions. The second part of the questionnaire contained questions relating to safety measures in the workplace and contained 11 questions. The third part examined incident situations at the examinees' workplace and it contained 11 questions, while the final (fourth) part dealt with examinees' vaccination status and encompassed eight questions.

The examinees were tested after the survey, i.e. their blood was taken to determine anti-HBs antibodies at the Laboratory for Virus Examinations of the Centre for Laboratory Medicine, Clinical Centre of Vojvodina, on a MINI VIDAS (bioMerieux, Marcy-l'Étoile, France) apparatus using the enzyme-linked fluorescence assay method.

The study involved workers engaged in immediate care and treatment of patients in tertiary health protection, with at least one year of service and voluntary consent for participation in the research. The research was approved by the Ethics Council of the Clinical Centre of Vojvodina on January 29, 2019 in the Consent Decision No.00-52.

The IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) program package was used for statistical data processing. Methods of descriptive and interferential statistics were used for data analysis. Numerical marks with normal placement were described using the arithmetic mean and standard deviation (SD).

The existence of correlation between variables was examined with the Spearman's rank correlation, and the strength of bonds was determined with guidelines provided by Cohen (small correlation r = 0.1–0.29; medium correlation r = 0.3–0.49; large correlation r = 0.5–1) [15].

RESULTS

The average age of the examinees was $\bar{x}$ = 43.13 years (SD = 10.22 years), the average height $\bar{x}$ = 168.17 cm (SD = 7.83 cm), the average body weight $\bar{x}$ = 70.59 kg (SD = 14.44 kg). The examinees had an average of $\bar{x}$ = 18.83 years of service (SD = 10.15 years).

Ninety examinees (90%) were female, and 10 (10%) were male.

The examinees were mostly medical nurses/technicians employed in internal medicine fields, who perform their tasks in wards, working in shifts (Table 1).

Table 1. Structure of the examinees in relation to their workplace

Branch of medicine	n	%
Surgery	13	13
Internal medicine	66	66
General medicine	21	21
Occupation		
Specialist doctor	15	15
Doctor	5	5
Registered nurse	12	12
Nurse with bachelor's (appl.) Degree	7	7
Nurse	61	61
Workplace		
Ambulance	18	18
Ward	75	75
Intensive / semi-intensive care unit	7	7
Shift work		
Yes	54	54
No	46	46
Total	100	100

Vaccination coverage in the observed sample was 97%. Completed HBV vaccination was listed by 87% of the examinees, majority of whom indicated that the time passed from the vaccination was 5–10 years. Testing for BBD during employment was reported by 41% of the examinees (Table 2).

Out of the total number of the examinees, 7% did not have anti-HBs antibodies for HBV infection, i.e. their values were lower than 10 mIU/ml (Table 3).

Table 2. Examinee structure according to the vaccine status (coverage) against HBV infection

Have you been vaccinated against HBV?	n	%
Yes	97	97
No	3	3
Are you completely vaccinated against HBV?		
Yes	87	87
No	13	13
When were you vaccinated against HBV?		
A year ago	9	9
Between five and 10 years ago	51	51
More than 10 years ago	37	37
I am not vaccinated	3	3
Were you tested for BBD during employment?		
Yes	41	41
No	45	45
I do not remember	14	14
Do you have HBV infection?		
Yes	0	0
No	100	100
Total	100	100

Table 3. Examinee structure by anti-HBs antibodies values

Anti-HBsAt	Min.		Max.	$\bar{x}$	SD
	3–10 (7)	11–500 (63)	≥ 500 (30)	247.94	199.704

Carrying out medical tasks in which they come into contact with blood and other patients' bodily fluids was stated by 90% of the examinees, out of whom 77% believed that they have been exposed to a constant HBV infection risk (Table 4).

Table 4. Examinee structure according to the degree of exposure to HBV infection and the number of workplace incidents

Exposure to infection risk	n	%
Yes	77	77
No	4	4
Periodically	19	19
Contact with biological material		
Yes	90	90
No	10	10
Needle prick		
Yes	36	36
No	64	64
Injury by a sharp object		
Yes	34	34
No	66	66
Contact with blood through the skin		
Yes	53	53
No	47	47
Contact with blood through the mucous membranes		
Yes	23	23
No	77	77
Total	100	100

The incident situation at workplace mentioned most frequently by the examinees was exposure to patients' biological material through skin (53%) and by needle prick (36%) (Table 5).

Table 5. Review of mean values for the number of workplace incidents

Workplace incident	n	$\bar{x}$	M	Min.	Max.	SD
Needle prick	36	3.78	2.5	1	20	3.78
Injury by a sharp object	34	5.21	5	1	20	4.48
Exposure through the skin	53	7.34	4	1	110	14.94
Exposure through the mucous membrane	23	4.91	3	1	20	4.69

Safety measures while working with patients are applied by 89% of the examinees. In regard to the safety measures the examinees use in their workplace during care and treatment procedures, the highest percentage of examinees specified the use of protective gloves (88%), while the use of safety glasses was reported by the lowest percentage of the examinees (24%).

Eighty-seven examinees (87%) confirmed that the employee safety was carried out continuously in their institution (Table 6).

Table 6. Examinee structure according to the use of protection measures in the workplace

Using protection measures while working with patients	n	%
Yes	89	89
No	11	11
Use of gloves		
Almost never	4	4
Seldom	5	5
Always	88	88
Only when I know that a patient has an infectious disease	3	3
Use of a mask		
Almost never	8	8
Seldom	21	21
Always	60	60
Only when I know that a patient has an infectious disease	11	11
Use of safety glasses		
Almost never	52	52
Seldom	15	15
Always	24	24
Only when I know that a patient has an infectious disease	9	9
Protection of workers from HBV in your institution is enforced		
Continuously	87	87
Sporadically	11	11
I am not informed	2	2
Total	100	100

An equal percentage of the examinees (92%) was aware of the meaning of the PEP term and of the department they need to contact for help after being exposed. Incidents in the workplace as adverse events were mentioned by 22% of the examinees, while 97% disposed of infective waste according to the rules of profession (Table 7).

Correlation of certain variables was examined with the aim to determine the relation between age, sex, body mass index (BMI), field of medicine in which the examinees work, workplace and years of service with certain factors affecting the health workers' protection from HBV infection (vaccination completeness, titer HBs antibodies, exposure to the risk of infection, number of interventions, the use of protective equipment, number of incidents, and more) (Table 8).

Table 7. Examinee structure according to the degree of familiarity with the procedures that affect the protection in the workplace and PEP measures

Existence of instructions for the protection of employees in the workplace	n	%
Yes	91	91
No	2	2
I am not sure	7	7
Reporting incidents as adverse events		
Yes	22	22
No	29	29
I am not sure	19	19
Knowledge of the term PEP		
Yes	92	92
No	3	3
I am not sure	5	5
Knowledge of post exposure procedures		
Yes	86	86
No	8	8
I am not sure	6	6
Knowledge of post-exposure help services		
Yes	92	92
No	4	4
I am not sure	4	4
Disposal of infectious waste in accordance with the rules of the profession		
Yes, always	97	97
No, never	2	2
From time to time	1	1
Total	100	100

Years of age have a weak positive statistically significant correlation with testing during employment (older employees were seldom tested during employment), and the time passed from vaccination (with older examinees more time passed since vaccination).

The sex variable has a low statistically significant correlation with testing during employment (female examinees are tested more often) (Table 8).

Table 8. Significant correlations of the examined variables

Variable	Variable	ρ	p
Age	employment testing	0.206	0.039
	number of interventions	-0.224	0.025
	knowledge of the term PEP	-0.303	0.002
	time elapsed since vaccination	0.243	0.015
Sex	employment testing	0.211	0.035
	vaccination completeness	-0.246	0.014
BMI	knowledge of the term PEP	-0.271	0.006
	anti-HBs antibodies	-0.206	0.040
Branch of medicine	exposure to the risk of infection	0.208	0.037
	performing interventions	0.245	0.014
	spraying blood into the eye or other mucosa	0.243	0.015
	reporting incidents as adverse events	0.349	0.003
	disposal of infectious waste according to the rules of the profession	0.287	0.004
	testing for BBD	-0.385	0.000
Occupation	shift work	0.288	0.004
	performing interventions	-0.253	0.011
	number of interventions in 24 h	0.433	0.000
	use of protective equipment	-0.364	0.000
Workplace	testing for BBD	-0.239	0.016
	exposure risk from BBD	-0.230	0.022
	disposal of infectious waste according to the rules of the profession	-0.203	0.43
	anti-HBs antibodies	0.242	0.015
Years of service	knowledge of BBD	0.232	0.020
	employment testing	0.216	0.031
	number of incidents	-0.237	0.018
	knowledge of the term PEP	0.235	0.019
	time elapsed since vaccination	0.321	0.001

BMI – body mass index; PEP – post-exposure prophylaxis; BBD – blood-borne diseases

DISCUSSION

Due to disease risks of health professionals, vaccination for HBV infection is mandatory in most countries. However, health workers' vaccination ranges from 15% in Africa to 75% in Australia, the USA, and New Zealand [16].

Studies conducted in Nigeria, China, Tanzania, and the USA show that health workers' vaccination scope for HBV infection ranges from 18% (Nigeria) to 84% (USA) [17–20].

In our country, health care professionals are subject to mandatory hepatitis B immunization according to epidemiological indications since 1989.

An epidemiological study was carried out in the Republic of Serbia on the territory of Nišava and Toplica districts (2000–2009), which determined that the health workers' vaccination coverage for hepatitis B was 31% [21].

In December 2015, research was carried out for the predictors of vaccination status connected with immunization for hepatitis B with persons working at the Clinical Centre of Serbia (Belgrade) in a cross-sectional study. The prevalence of vaccination in the examined sample was 66% [22].

Our research with HCWs employed in a facility of tertiary health care showed a high vaccination coverage of 97%. The obtained results show an increase in the coverage degree, and the fact that important steps are taken in educating HCWs on the protection from BBD.

When it comes to personal protection, 89% of examinees used protective measures when performing professional duties and these most often included gloves (88%), while protective glasses were used least frequently 24%.

In the Republic of Serbia, a research was carried out on the territory of the Autonomous Province of Vojvodina related to the importance of blood-borne infection prevention and control for the decrease of professional risks amongst HCWs. The research results showed that health professionals in Vojvodina have a high rate (more than 80%) of professional exposure to these infections [23]. The same was confirmed by our research, *viz.* 90% of examinees carry out medical tasks during which they come into contact with patients' biological waste.

Some countries (Sudan) recognized the need to study the exposure of HCWs to HBV infection in the workplace when performing care and treatment activities. The obtained results showed a high level (above 65%) of infection exposure [24].

WHO estimates that every year around 66,000 health care professionals are infected with HBV, and 600,000–800,000 health professionals experience an incident in the workplace in the form of a cut or a needle prick [14, 25].

Our research shows that the highest exposure is suffered by HCWs who perform numerous medical tasks i.e. interventions during the day, and it is the nurses/technicians who are more exposed than other examinees. It is exactly the nurses/technicians who reported the highest number of incidents while working. The research carried out showed that younger nurses-technicians, especially those with higher BMI, have more frequent contacts with blood and other patients' bodily fluids (Table 8).

The aim of research conducted in China, India, Japan and Catalonia was to determine the level of health care professionals' protection against HBV infections after vaccination procedure has been completed. The acquired data indicate that the protection efficiency ranges between 64% (Catalonia) and 83% (Japan), i.e. these are the percentages of examinees with protective anti HBs antibodies [26–29].

Even though there is permanent seroprophylaxis at the Clinical Centre of Vojvodina, 7% of the examinees involved in this research did not have anti-HBs antibodies (< 10 mIU/ml), which implies that the protection efficiency for HBV infection in the monitored sample is 93%. All seven examinees who did not have a protective antibody titer were vaccinated with three doses of the vaccine. In four examinees, the time elapsed since the last of vaccine was one year, in two examinees between five and 10 years, and in one more than 10 years.

Among factors that influence the level of anti-HBs antibodies, our study confirms the influence of BMI, because it has a low negative correlation to anti-HBs antibodies (the higher the BMI, the lower the anti-HBs antibodies – Table 8).

Higher exposure to infection and lower level of response to the vaccine with examinees who have a higher BMI is explained through certain metabolic disorders that change the immune system's response and thus contribute to the increased sensitivity to bacterial, viral, or fungal infections [30].

Younger examinees were more familiar with the meaning of the PEP term, and the procedure after being exposed to a workplace incident, even though 86% gave a positive reply to a question about their knowledge of the post-exposure procedure. Knowledge on prevention and control of BBD and PEP should be implemented into school curriculums of vocational schools and faculties. It is the basis for acquiring knowledge and skills, which should be improved from the moment of employment for every health worker and then continued during the entire working life.

CONCLUSION

The conducted research showed high average HBV infection vaccination amongst HCWs (97%), as well as high level of protection 93%. Health workers' exposure in the observed tertiary health care institution was 90%. Safety measures against HBV infection were carried out by HCWs in 89% of the cases, whereas 86% of employees responded positively about being familiar with the PEP term.

Considering the fact that, in our country, there is no valid nor complete data on HCWs' vaccination coverage for HBV infections, on the protection level of employees who underwent immunization, on the number and the type of incidents in the workplace and the PEP applied, activities of all relevant institutions in the country should be guided towards solving this increasing problem.

Conflict of interest: None declared.

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Заштита здравствених радника запослених у терцијарној здравственој установи од инфекције вирусом хепатитиса Б

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САЖЕТАК

Увод/Циљ Вирусом хепатитис Б (ВХБ) инфицирано је више од 300 милиона људи широм света. Преноси се путем крви, крвних деривата, сексуалним путем и вертикално, а здравствени радници због природе свог посла представљају вулнерабилну групу.

Циљ овог истраживања био је да се утврди обухват вакцинацијом против инфекције ВХБ здравствених радника радно ангажованих у терцијарној здравственој установи –Клиничком центру Војводине, ниво те заштите одређивањем титра анти ХБс антитела, степен изложености, степен поштовања мера заштите на радном месту испитаника и степен познавања мера постекспозиционе профилаксе (ПЕП).

Методе Истраживање је спроведено као дескриптивна аналитичка студија пресека, у којој је као инструмент истраживања коришћен Упитник о заштити здравствених радника против крвнотрансмисивних болести, а узоркована је крв за одређивање титра анти-ХБс антитела. Узорак је чинила једна стотина здравствених радника.

Резултати Истраживање је показало висок ниво обухвата вакцинацијом против инфекције ВХБ (97%). Поред континуиране серопрофилаксе одређен број испитаника нема заштитни титар антитела (7%). Ниво експозиције здравствених радника настанку инфекције ВХБ износи 90%. Заштитне мере на радном месту користи 89% испитаника, док 86% познаје мере ПЕП.

Закључак Истраживање је показало висок ниво обухвата здравствених радника специфичном заштитом против инфекције ВХБ, недовољан степен спроведене заштите, висок ниво изложености настанку инфекције ВХБ, непотпуно поштовање мера заштите и недовољно познавање мера ПЕП.

Кључне речи: титар антитела; здравствени радници; хепатитис Б; акциденти; заштита на раду

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Is age-adjusted Modified Early Warning Score upon admission a relevant prognostic tool for final outcome?

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SUMMARY

Introduction Early warning scoring systems are important for timely identification of the critically ill, but are they a relevant prognostic tool? Our objective was to test if Modified Early Warning Score (MEWS), lactate, and base excess (BE) have any prognostic value in high dependency unit patients.

Methods This was a prospective observational study that included 364 patients treated at a respiratory high dependency unit. The values of MEWS, lactate, and BE at admission were recorded with patients' age, sex, and comorbidities. Negative outcome was defined as death or transfer to the intensive care unit. Independent predictors of negative outcome were identified with the use of multivariable logistic regression.

Results Of 369 patients, 203 (55%) were male. Mean age was 62 ± 16. There were 138 (37.4%) patients with negative outcome: 27.37% died, while 10.03% patients required intensive care unit transfer. The median length of hospital stay was 13 days (IQR 7–15). Patients with negative outcome had a significantly higher MEWS (3.68 ± 1.965 *vs.* 4.57 ± 2.33, $p < 0.001$), lower BE (-0.139 ± 7.48 *vs.* -3.751 ± 6.159, $p < 0.001$), and a higher lactate (2.299 ± 2.350 *vs.* 3.498 ± 3.578, $p < 0.001$). MEWS ≥ 4 (OR 1.90, CI 1.082–3.340, $p = 0.026$) was the only independent predictor of mortality. Area under the curve (AUC) for MEWS with regard to in-hospital mortality prediction was 0.633 (95% CI 0.569–0.697). When age was added to MEWS, the AUC was 0.76 (95% CI 0.707–0.814).

Conclusion Our findings support the prognostic value of MEWS for final outcome of patients admitted to the high dependency unit.

Keywords: MEWS; lactate; BE; outcome

INTRODUCTION

Various versions of early warning scores (EWS) are proposed for timely identification of the critically ill [1–4]. The ultimate goal is to timely recognize clinical deterioration, which facilitates early intervention. One of the wide spread scores in clinical practice is the Modified Early Warning Score (MEWS) [5, 6, 7]. In the most recent study, EWS were also proposed as a prognostic tool, but further validation is necessary [8]. Addition of laboratory findings to increase the value of clinical scores has been considered [9–22]. Since our respiratory high dependency unit (HDU) is mainly used for treating patients diagnosed with pneumonia and sepsis, severe chronic obstructive pulmonary disease exacerbation, and pulmonary thromboembolism, we decided to test lactate, base excess (BE), and age in addition to MEWS, as predictors of final outcome.

METHODS

This study was prospective and observational. It took place at the respiratory HDU of the Institute for Pulmonary Diseases. The study was done in accordance with the Committee on Ethics of the Institute for Pulmonary Diseases of Vojvodina. During the time period from 2009 to 2014, the following data were recorded for 369 patients: age, sex, comorbidities, vital signs and the calculated MEWS at admission, as well as lactate and BE at admission, length of stay, and outcome. There were 501 patients treated at the respiratory HDU during the given time period; however, due to technical issues, it was not possible to measure lactatemia in 132 patients, and they were omitted from the study. The negative outcome was either intensive care unit (ICU) transfer or death, and the positive outcome was discharge from the hospital or transfer to the general ward. We used the following cut-off values: MEWS ≥ 4, lactate ≥ 2.5 mmol/1, age ≥ 65years and BE < -2 mmol/l.

We used percentages to present categorical variables and their comparison was performed with the help of either Fisher's exact test or χ^2. Either mean (± SD) or median (interquartile range – IQR) were used to present continuous variables and the values were further compared using Student's t-test or the Mann–Whitney U-test. Odds ratios between individual factors

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and the mortality were calculated with univariate logistic regression, followed by multivariable logistic regression in order to recognize independent mortality predictors. Sensitivity and specificity at the given cut-off of ≥ 4 points were determined for MEWS score, followed by the receiver-operating characteristic (ROC) curve.

RESULTS

The mean age of 369 patients was 62 (± 16) years. There were 215 (58.3%) male patients. The leading diagnosis at admission was pneumonia for 151 patients (40.92%). As many as 341 (92.4%) had at least one comorbidity – mostly cardiovascular. Age, sex, co-morbidities, and initial diagnosis upon admission for all patients are listed in Table 1.

No difference was found in MEWS values between the patients with and without co-morbidities (Table 2).

Table 1. Baseline characteristics of the patients

Variables		n (%)
Sex	Male	215 (58.3%)
	Female	154 (41.7%)
Comorbidities	Cardiovascular	260 (70.5%)
	Respiratory	143 (38.8%)
	Neurological	73 (19.8%)
Age	< 65	180 (48.8%)
	≥ 65	189 (51.2%)
Diagnosis at admission	Pneumonia	151 (40.92%)
	Chronic obstructive pulmonary disease	78 (21.1%)
	Sepsis	59 (16%)
	Pulmonary embolism	28 (7.6%)
	Respiratory failure in neurological diseases	8 (2.17%)

Table 2. Modified Early Warning Score in patients with and without comorbidities

Comorbidities	< 4		≥ 4		Total	
	n	%	n	%	n	%
Without	13	7.5%	15	7.7%	28	7.6%
With	160	92.5%	181	92.3%	341	92.4%
Total	173	100%	196	100%	369	100%

Initial MEWS was taken in all the patients, as well as lactate, BE, and the length of stay. All the values were compared between the groups with positive and negative outcome. Two hundred thirty-one (62.6%) patients had the positive outcome. Patients with the negative outcome had a significantly higher MEWS (3.68 ± 1.965 *vs.* 4.57 ± 2.33, p < 0.00l), lower BE (-0.139 ± 7.48 *vs.* -3.751 ± 6.159, p < 0.001), and a higher lactate (2.299 ± 2.350 *vs.* 3.498 ± 3.578, p < 0.00l).We found no difference in the length of stay between the groups with different outcome (17.00 ± 11.697 *vs.* 14.44 ± 18.709, p = 0.106).

We correlated initial MEWS with lactatemia and found a weak positive correlation (r = 0.245, p < 0.001).

We also compared initial MEWS with BE and found a weak positive correlation (r = 0.202, p < 0.001).

Median length of hospital stay was 13 days (IQR 7–15). We did not find that patients with MEWS ≥ 4 had more hospital days (17.00 ± 11.697 *vs.* 14.44 ± 18.709, p = 0.61). Odds ratio between individual factors and mortality were calculated with univariate logistic regression, and the identified factors that had a correlation with mortality were the following: MEWS ≥ 4 points, lactate ≥ 2.5 mmol/l, BE < -2 mmol/l, as well as the age ≥ 65 and the presence of comorbidities (Table 3).

In the following step potential independent mortality predictors were identified with the use of multivariable logistic regression – the results are shown in Table 4. Multivariate logistic regression showed that MEWS and age were independent mortality predictors. The strongest predictor of mortality was MEWS with OR of 1.9.

Table 3. Univariate logistic regression model to estimate unadjusted odds ratios between each factor and mortality

Variables	OR	95% CI	p
Modified Early Warning Score ≥ 4	2.119	1.296–3.465	0.003
Lactate ≥ 2.5	2.477	1.531–4.008	< 0.001
Base excess < -2mmol/l	2.579	1.68–4.516	< 0.001
Age ≥ 65	1.069	1.046–1.093	< 0.001
Comorbidities	4.732	1.101–20.337	0.037

Table 4. Multivariate logistic regression analysis showing independent predictors of mortality

Variables	Cut-off values	p	OR	95% CI
MEWS	≥ 4	0.026	1.901	1.082–3.340
Lactate	≥ 2.5	0.173	1.479	0.842–2.591
BE	< -2 mmol/l	0.06	1.173	1.000–3.142
Age	> 65	< 0.001	1.058	1.034–1.082
Comorbidities	Present	0.348	2.262	0.412–12.433

MEWS – Modified Early Warning Score; BE – base excess; OR – odds ratio; CI – confidence interval

The area under the curve (AUC) for MEWS was 0.633 (95% CI 0.57–0.7). The model which included the age and MEWS (AUC 0.76, 95% CI 0.707–0.814) was superior to MEWS alone (AUC 0.633, 95% CI 0.569–0.697). The calculated AUC for BE was only 0.338 with 95% CI 0.272–0.404 and AUC for lactate was 0.652 with 95% CI 0.585–0.718. The addition of both lactate and BE to the model which included MEWS and age did not improve the AUC (AUC 0.79, 95% CI 0.74–0.843).

DISCUSSION

The rationale behind the use of EWS is quite straightforward – their crucial clinical role lies in timely recognition of clinical deterioration on the ward. Acute deterioration is most frequently preceded by changes in vital parameters, which constitute EWS [1–10]. In this study, we confirmed the predictive value of MEWS and age in identifying HDU patients at high risk for death or ICU admission. Further addition of BE and lactate were not found to improve the outcome prediction.

Alam et al. [3] presented the results of systematic review on impact of EWS on patient outcomes. Seven large studies

were included, but meta-analysis was not possible due to heterogeneity. They concluded that there was a positive trend towards improved outcomes after EWS were introduced. The main limitation of this review was the fact that no single standardized EWS was used. One of the best validated variants of EWS is the MEWS. Implementation of this score has shown reduction in hospital mortality, number of ICU days and number of adverse events [5, 6, 7]. When our respiratory HDU was established in April of 2009, we choose to incorporate MEWS in the chart. One of the aims was to demonstrate its effectiveness in every day practice in order to introduce it to our general wards without too much resistance from the already overburdened staff.

The study was prospective and observational in design, but it has several limitations. The first limitation is that we excluded 132 patients due to the fact that our laboratory could not perform lactate testing at all times. Second limitation is that "initial" MEWS, along with lactate and BE, refers to the values measured upon admission to the respiratory HDU – more than half of the patients were transferred from the ward, while the rest were admitted directly to the HDU. Another limitation is that comorbidities were noted but Charlson comorbidity index was not calculated in order to better classify their burden and severity.

We found that 341 (92.4%) patients had at least one comorbidity, but there was no difference in initial MEWS values between the groups with and without comorbidities. In the study by Çıldır et al. [23] there was a significant difference between surviving patients and those who died, in both MEWS values and Charlson comorbidity index, but the two indices were not compared to each other.

Initial MEWS values were compared between the groups with different outcome. Due to the specific role of the HDU, we defined the positive outcome as either transfer to the ward or discharge from the hospital, while death and transfer to the ICU were defined as the negative outcome. A total of 231 (62.6%) patients had the positive composite outcome, and patients with the negative composite outcome had a significantly higher MEWS. This finding is in accordance with the results of Goldhill et al. [1] – they conducted a study on 1047 patients, in which they concluded that an increasing EWS was associated with higher hospital mortality. Burch et al. [5] conducted a study on 790 patients and they also found that increasing MEWS was associated with higher rates of intrahospital mortality. Similarly, EWS were previously tested as potential predictors of serious adverse events in hospitals. Ludikhuize et al. [6] performed a study which included 204 patients. They found that 81% patients had MEWS score three points or higher on at least one occasion during the 48-hour period preceding the adverse event. Recently, Liu et al. [2] performed a cohort study in patients with and without the infection comparing five EWSs regarding their potential role to predict in-hospital mortality and the combined outcome of ICU transfer or mortality. National Early Warning Score (NEWS) and MEWS had the highest discrimination power to predict the outcome in comparison with the Quick Sequential Sepsis-Related Organ Failure Assessment (qSOFA), and Systemic Inflammatory Response Syndrome (SIRS) [2].

The median length of hospitalization in our study was 13 days [IQR 7–15]. We did not find that patients with initial MEWS ≥ 4 had a longer length of stay. Also, we found no difference in the length of hospitalization between the groups with the positive and the negative outcome. In a large study for MEWS validation, Subbe et al. [7] showed that 7.1% of all patients had MEWS ≥ 5 at admission, compared to only 1.8% on the third day. However, in a recent study by Kruisselbrink et al. [4] in a resource-limited setting, the median duration of hospitalization was nine days. The authors found a much higher percentages of MEWS ≥ 5 after a median of nine days. Torsvik et al. [24] conducted a post-intervention study in a Norway hospital on 409 patients, and the intervention included introduction of a flow chart for sepsis identification including all vital parameters, doctors' response time, and treatment. They found that the length of stay was 3.7 days shorter after the intervention. The explanation is that timely identification of high-risk patients leads to earlier intervention and/or shorter delay to ICU transfer. However, in a study by Paterson et al. [25], the results showed that the length of stay extended significantly in relation to increasing the EWS score, as well as that the EWS score of ≥ 4 resulted in doubling of the hospitalization length. Similarly, Groarke et al. [26] found that higher admission EWS correlated with longer hospital stay.

In our study, risk factors for higher mortality in the univariate analysis were the following: MEWS ≥ 4 points, lactate ≥ 2.5 mmol/l, BE < -2 mmol/I, the presence of comorbidities, and the age of ≥ 65.

Multivariable logistic regression analysis identified two independent mortality predictors – MEWS and age. In the study by Jacques et al. [2], BE of less than -5 mmol/1 was also confirmed as a predictor of serious adverse events. Groarke et al. [26] found that admission EWS can be a valuable score for triage in acute medical admissions – they concluded that there was a higher risk for ICU admission, as well as death for each rise in the EWS category. Paterson et al. [25] designed a study to assess effects of a standardized EWS on patient outcomes in acute admissions – they included 848 patients, both medical and surgical. The results confirm that high admission EWS indicated higher risk of hospital mortality. Moreover, the medical staff filled a questionnaire where they indicated the use of a scoring system helped detect illness severity (80%) which prompted earlier interventions (60%). One of the most significant early studies for MEWS validation by Subbe et al. [7] found that MEWS of ≥ 5 points correlated with increased risk for mortality as well as ICU admission. Kruisselbrink et al. [4] found that MEWS above four points was associated with increased mortality. However, the most recent argument in favor of MEWS is the study by Churpek [8], whose results were published in 2016. The study compared four different scores in order to determine their value in predicting hospital mortality and transfer to the ICU. The scores were MEWS, qSOFA, NEWS, and SIRS. The study included 30,677 patients who first met the criteria for suspected infection from 2008 to 2016. The results show that NEWS was the best predictor of hospital mortality, and

MEWS was the second best. Authors concluded that the newly proposed qSOFA score was not a good substitute for EWS when it comes to identifying high-risk patients with suspected infection. Another study published in 2016, by Wang et al. [27], established that peri-arrest MEWS values predicted the outcome. On the other hand, an Italian study published in 2017 performed on 526 patients with sepsis states that even though increasing MEWS correlated with mortality, AUC did not show that MEWS had a sufficient sensitivity for predicting in-hospital mortality [28]. Mitsunaga et al. [29] showed that NEWS and MEWS predict hospital mortality in the elderly.

There are studies in which addition of biochemical markers increased the AUC for predicting intra-hospital mortality. Perera et al. [30] found that MEWS of ≥ 5 points, along with increasing age, predicted outcome. In order to increase the sensitivity of prediction, they suggested a combined score consisting of MEWS and several biochemical parameters: CRP, albumin, and platelet count. Ho et al. [11] showed that combining plasma lactate with qSOFA score significantly increases the ability to predict mortality in patients with infection [11]. Our study did not demonstrate additional benefit of adding BE and lactate level to the age and MEWS in predicting mortality risk in HDU patients. It is possible that this is due to heterogeneity of the population – we included patients with pneumonia, sepsis, but also acute chronic obstructive pulmonary disease exacerbation and pulmonary thromboembolism. Further research in each of these subgroups may show different results.

CONCLUSION

The findings of our study suggest that the MEWS, adjusted for age, represents a valuable prognostic tool for final outcome and an independent predictor of hospital mortality for HDU patients. According to the recent studies about the significance of EWS to predict outcome in hospitalized patients, the results of our study are another contribution to use them for identifying the patients who are at risk for in-hospital death or who are in need of transfer to the ICU.

Conflict of interest: None declared.

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Да ли према старости коригована вредност скора *MEWS* при пријему има прогностичку вредност у односу на коначан исход лечења?

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САЖЕТАК

Увод Бодовни системи за рано препознавање су важни за идентификацију критично оболелих, али да ли су и прогностички алат? Циљ је био проверити прогностичку вредност модификованог раноупозоравајућег бодовног скора (*MEWS*), лактата и базног ексцеса (БЕ) код болесника примљених у јединицу полуинтензивне терапије.

Методе Проспективна опсервациона студија обухватила је 369 болесника хоспитализованих у пулмолошку јединицу полуинтензивне терапије. Вредности *MEWS* скора, лактата и БЕ при пријему забележене су, као и доб болесника, пол и присуство коморбидитета. Негативни исход је дефинисан као смрт или премештај у јединицу интензивног лечења. Фактори за које је униваријантном анализом утврђена статистичка значајност анализирани уз помоћ мултиваријантне логистичке регресије, у циљу утврђивања независних предиктора неповољног исхода.

Резултати Од укупно 369 болесника, 203 (55%) су били мушкарци, а просечна старост је била 62 ± 16 година. Неповољан исход лечења забележен је код 138 (37,41%) болесника: 27,37% је умрло; а 10,03% болесника премештено је у јединицу интензивног лечења. Просечна дужина хоспитализације била је 13 дана (*IQR* 7–15). Болесници са неповољним исходом имали су значајно веће вредности *MEWS* (3,68 ± 1,965 *vs.* 4,57 ± 2,33, $p < 0,001$), нижи БЕ (-0,139 ± 7,48 *vs.* -3,751 ± 6,159, $p < 0,001$), и виши лактат (2,299 ± 2,350 *vs.* 3,498 ± 3,578, $p < 0,001$). *MEWS* ≥ 4 (*OR* 1,90, *CI* 1,082–3,340, $p = 0,026$) се издвојио као једини независни предиктор морталитета. Површина испод криве (*AUC*) за *MEWS* у функцији предиктора морталитета била је 0,633 (95% *CI* 0,569–0,697). Корекцијом у односу на старост болесника, *AUC* је била 0,76 (95% *CI* 0,707–0,814).

Закључак Резултати студије потврђују прогностичку вредност *MEWS* бодовног система у односу на коначан исход лечења болесника јединице полуинтензивног лечења.

Кључне речи: *MEWS*; лактат; БЕ; исход

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Glucose concentration monitoring using near-infrared spectrum of spent dialysis fluid in hemodialysis patients

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SUMMARY
Introduction/Objective Diabetic nephropathy leading to end-stage renal disease is a major health problem worldwide. Hemodialysis (HD) treatment is associated with glycemia variations. Diabetic patients on HD might benefit from a non-invasive online glycemia monitoring system.
The aim of this study was to assess the glucose concentration from the matrix of the spent dialysate fluid using near-infrared (NIR) spectroscopy.
Methods Blood samples and spent dialysate were collected in the 15th minute of the HD treatment from 15 patients. The spent dialysis fluid was characterized by a NIR spectrometer in the range of 900–1300 nm. In order to apply the artificial neural network (ANN) and train it, the MATLAB NFTOOL program was used. The testing and training of the ANN were executed using the NIR spectrum of the spent dialysis fluid as input, and the glucose concentration as output.
Results A significant correlation in excess of 93% between the NIR spectrum of the spent dialysate and the blood glucose concentration (3–9 mmol/l) was found.
Conclusions NIR spectroscopy is a non-invasive and reliable method of glycemia monitoring which can be used in maintaining HD patients.
Keywords: hemodialysis; machine learning; spent dialysate; VIS-NIR; patient-specific

INTRODUCTION

Chronic kidney disease and diabetes mellitus are public health problems that influence millions of people all over the world. The latest estimates from the International Diabetes Federation suggest that there were 415 million diabetes mellitus patients in 2015 and that there will be 642 million by 2040 [1]. Inadequate blood glucose control is considered the major cause of diabetic nephropathy and the progression of renal insufficiency, eventually leading to end-stage renal disease requiring renal replacement treatments – either transplantation or dialysis.

The most-studied biological fluids of clinical interest are blood, urine, and, recently, spent dialysate. The dialysis fluid is obtained by mixing water for dialysis with an electrolyte concentrate in a dialysis machine. This machine guarantees the electrolytic composition, the pH, temperature, and the flow rate of the dialysis liquid. Heise et al. [2] gave a complete overview of biological fluids that can be explored using the near-infrared (NIR) spectroscopy. Eddy and Arnold [3] have shown the possibility of glucose detection using NIR spectroscopy.

Hemodialysis (HD) patients with diabetes mellitus must undergo frequent controls of glycemia. Standard monitoring methods are uncomfortable, invasive, and painful. In addition, they only give the interstitial glucose level. Furthermore, it has been shown that the blood glucose levels vary during the HD treatment. During the procedure, the glycemia tends to decrease, while it increases when the HD session ends. Thus, at least for HD diabetic patients, a non-invasive, painless, on-line glycemia monitoring would be beneficial as both hypo- and hyperglycemia should be avoided [4].

However, on-line monitoring of suppressants such as urea, creatinine or blood glucose is complicated by the fact that blood is a highly saturated fluid, prone to clotting [5, 6]. Monitoring of the glucose in the spent dialysate makes the system more flexible. An optical sensor, which simply shines a beam of light through a fluid that contains glucose and uses the principle that the absorption pattern of near-infrared light can be quantitatively related to the glucose concentration may be a simple but effective solution.

Glycemic patterns are still hardly predictable, making it difficult to control blood glucose levels without a risk of hypoglycemia. It is important for clinicians to be aware that there are limitations of specific point-of-care glucose meters [7]. Different assays are used for the quantification of glucose; one of the most sophisticated methods is infrared spectroscopy.

Non-invasive methods for monitoring glucose level based on infrared spectroscopy were first invented during the 1990s [8]. Since then, a wide range of techniques has been developed

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for the non-invasive observation of glucose based on chemical, optical, and electrochemical techniques [9–12].

This development of non-invasive techniques was preceded by successful *in vitro* studies that were based on the determination of glucose in aqueous solutions, or whole blood by NIRS [13, 14, 15]. Studies were mainly based on the effects of glucose on certain secondary processes. One of the most famous examples is effect of glucose on the scattering properties of tissue. However, propagation of light through tissue is complicated by the heterogeneous nature of the tissue matrix, thus creating a problem [13].

To the best of our knowledge, there is no published work on automatic glucose level anomaly detection based on characterization by ultraviolet–visible–near-infrared spectroscopy (UV–VIS–NIR) of the spent dialysate.

METHODS

During the research, patients without diabetes were selected because they have insignificant blood glucose fluctuations. The goal was to detect even the smallest changes in glucose concentration. It is expected that the machine learning algorithm would detect greater changes in concentrations with greater accuracy. The maximum value of glucose recorded during the research was 15.7 mmol/l, which is outside the range of normal values in the blood, while the minimum value was 3.9 mmol/l. The study included 15 non-diabetic male patients with end-stage renal disease on HD. All HD treatments were performed under the standard protocol, including ultrafiltration rates prescribed to remove the interdialytic weight gain. Dialysis was performed using Dialog+ Adimea (B. Braun Avitum AG, Melsungen, Germany) machines. The dialysate contained Na^+ 138 mmol/L, Cl^- 110.5 mmol/l, K^+ 2 mmol/l, Ca^{++} 1.75 mmol/l or 1.5 mmol/l, Mg^{++} 1 mmol/l, CH_3COO^- 3 mmol/l, HCO_3^- 32 mmol/l, glucose 1 g/l. The mean dialysate flow was 500 ml/minute, and mean effective blood flow was 300 ml/minute. All the patients were dialyzed via arterio–venous fistulas using a two-needle system. The Ethics Committee of the Dr Dragiša Mišović – Dedinje University Hospital Center, where the study was performed, reviewed the study protocols and all patients provided an informed consent before participating.

Sample collection

Samples of spent dialysate were collected directly from the dialyzer outlet, 15 minutes after the beginning of the dialysis procedure. At the same time, blood samples were taken from the arterial blood line, before entering the dialysis circuit. For each sample, 15 ml of spent dialysate solution was collected into a container and stored at room temperature for approximately three hours before being transported to the research laboratory.

Sample analysis

Blood glucose was measured using the Dimension R×L Max (Siemens Healthcare GmbH, Erlangen, Germany) machine. The assay is based on the hexokinase method. VIS-NIR absorbance spectra of the samples were measured the day after the HD treatment. The absorption spectrum of each sample was measured three times. UV–VIS–NIR optical absorption spectra have been registered using the spectrometer Lambda 950 (Perkin Elmer, Waltham, MA, USA). The wavelength region of interest was 900–1300 nm, and the UV/VIS resolution was set to 2 nm. The instrument was connected to a PC running the Windows 7 operating system and was controlled by the Perkin Elmer UV WIN LAB Explorer. Serum glucose was measured using the Dimension RxLMax (Siemens Healthcare GmbH) machine. The assay is based on the hexokinase method. Glucose level above 6 mmol/L was considered hyperglycemic [16].

Machine learning methods

Here, in order to form the artificial neural network (ANN) and its training, the NFTOOL of MATLAB (The MathWorks, Inc., Natick, MA, USA) program was used. The neural network used for function fitting was a two-layer feedforward network, with a sigmoid transfer function in the hidden layer and a linear transfer function in the output layer.

The test set data have no effect on the training process and it provides an independent measure of network performance during and after training. The training starts with two and finishes with 1000 hidden neurons. The hidden-layer neurons are increased when network is not performing well. The optimum number of hidden layers was determined to be four. Training multiple times generates different results due to different initialization of connection weights and different initial condition.

The NIR spectrum of spent dialysis fluid is used as inputs and red blood parameters was taken as output. The NIR spectrum of the spent dialysis fluid was used as the input to the network, and the blood glucose concentration as the output. In the network, Bayesian regularization function is used for network training.

RESULTS

The best results were achieved using four hidden neurons. The Bayesian regularization algorithm was used for the training of the network. With these settings, the input vectors and target vectors were randomly divided into training (207 samples) and test (90 samples) sets.

The following regression plot displays the network outputs with respect to targets for training and test sets. If $R^2 = 1$, this indicates that there is an exact linear relationship between outputs and targets. If R^2 is close to zero, then there is no linear relationship between the outputs and targets. The correlation coefficient (R-value) measures the correlation between outputs and targets. The correlation was considered excellent if R^2 was > 0.95, very good if R^2 was > 0.9 and < 0.95, good if R^2 was > 0.6 and < 0.8, and poor if R^2 was < 0.6.

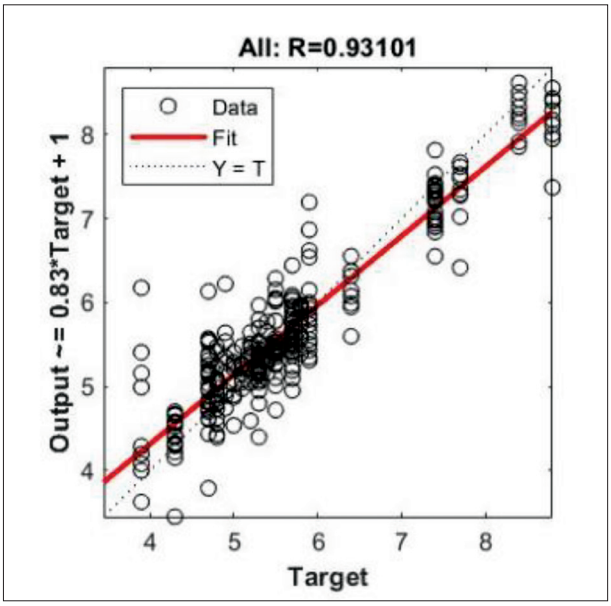

Figure 1. Regression plot between the near-infrared absorbance of spent dialysate and the glucose concentration in the patient blood during a hemodialysis session

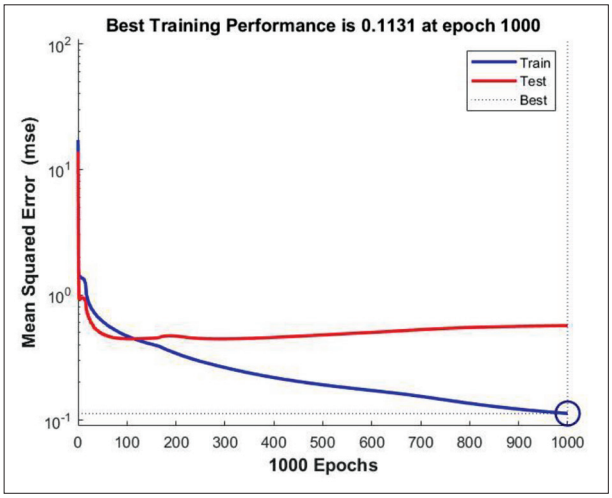

Figure 2. Train–performance plot: the mean squared error of the train and test data is shown against the training iteration number (epoch)

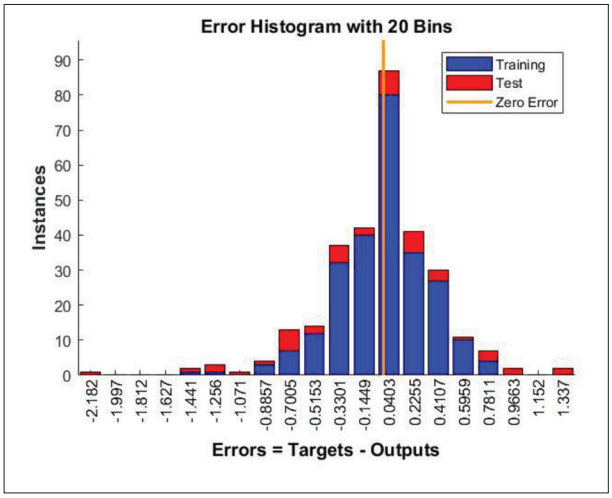

Figure 3. Error plot: the distribution of the difference between the training targets and network outputs for the training and test datasets

Figure 1 shows the regression plot between the NIR-absorbance of spent dialysate and the glucose concentration in the patient blood during an HD session (wavelength range 900–1300 nm, R^2 training = 0.96, R^2 test = 0.67, R^2 all = 0.93, number of spectra used for training was N = 270). The average glucose concentration in patients' blood was 5.72 ± 1.61 mmol/l. A good correlation of these data with the glucose levels in the patients' blood was confirmed by the analysis of discrete blood samples taken from arterial lines.

Figure 2 represents a plot of the train and test mean squared errors (MSE) with epochs. The best train performance was achieved at the epoch 1000, with the smallest MSE of 0.1131. The best test parameters were achieved at epoch 100. The equation relating the predicted and measured values is Output = 0.83 × Target + 1.

Figure 3 shows the distribution of the train and test errors for the trained network.

DISCUSSION

The prevalence of diabetes mellitus complications can be attenuated by adequate glycemic control pertinent to frequent blood glucose monitoring. Unfortunately, most of the available glucose measurement devices are invasive, making the procedure, which has to be repeated several times per day rather uncomfortable and painful. Besides this discomfort, diabetic patients on HD further undergo painful vein punctures every few days for dialysis treatment. Furthermore, there is evidence that HD treatment is associated with intradialytic hypoglycemia and postdialytic hyperglycemia [17]. Therefore, these patients would greatly benefit from a non-invasive intradialytic glucose monitoring.

NIR spectroscopy can be used as an alternative, non-invasive method for clinical analyses. In this method, NIR light is transmitted through or absorbed by the sample, and the substance concentration is predicted by analysis of the transmitted spectral information. Information about complex substances can be obtained from a single NIR spectrum [18]. Data obtained from the NIR spectrum of the spent dialysate fluid can be used for on-line monitoring of blood glucose concentration. The principle that the absorption pattern of NIR light can be quantitatively related to the glucose concentration has been confirmed in a number of previous studies [18–21].

Among all the available methods, the PLS regression has been used most widely for the analysis of NIR spectral data [18, 22]. The biggest problem with PLS methods is that the spectrum property relationship is supposed to be linear. However, this premise cannot be applied to systems with strong intermolecular or intramolecular interactions. If one measures the amount of glucose in a fluid that contains other substituents, the Beer–Lambert law cannot be applied because of interactions between components, an incorrect distribution of fluid components, and a baseline shift. All of these lead to a nonlinear system. This makes

non-linear calibration methods necessary for building robust calibration models since these methods have the potential to model heavy intrinsic non-linearities that can be found in natural multicomponent systems.

Machine learning has also been applied to non-invasive glucose measurements in various ways. This technology provides a way to improve the performance of a glucose monitoring system, and is used in optical, chemical, electrical, and microsensor techniques. The researchers have combined machine learning to investigate glucose levels in patients' blood [23, 24]. Machine learning methods have not only been applied in the tracking of glucose, but also in predicting hypoglycemia [25, 26, 27].

Here, in order to apply the ANN and train it, the MATLAB NFTOOL (The MathWorks, Inc.) program was used.

There are number of batch training algorithms that can be used to train a network, like Levenberg–Marquardt and Scaled Conjugate Gradient. In the network, Bayesian regularization function is used. This function updates the weight and bias values according to the Bayesian optimization method. The network was adjusted in the direction of reducing the error by iteration.

Further improvements in method precision might be expected with additional wavelength ranges, and by instrument improvements that will reduce or cancel noise.

It should be noted that the presented methodology has been shown to detect very subtle glucose variations in non-diabetic patients and that, therefore, this approach is expected to yield even more precise and reliable glucose readings in diabetic HD patients.

CONCLUSION

In this work, a new approach through machine learning and NIR spectroscopy of the spent dialysis fluid has been proposed to improve the fast prediction of blood glucose levels in HD patients. Neural networks have been demonstrated to be remarkably effective in terms of efficiency (training time) and performance ($R > 0.93$). The accuracy and precision of R, for the determination of the concentration of blood glucose obtained using the NIR spectrum of spent dialysis fluid is enough to be useful as a diagnostic screening method. The results confirmed this is a safe, accurate, reliable, and non-invasive method to assess glycemia during HD treatment. The chosen methodology renders its application useful for other pharmacokinetic and pharmacodynamic problems. Further studies on larger patient cohorts would provide valuable results that could be used to design built-in or plate glycemic sensors for dialysis machines. Moreover, machine-learning methods can be used to upgrade the current software in dialysis machines.

Conflict of interest: None declared.

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Праћење концентрације глукозе у крви болесника на хемодијализи коришћењем отпадног дијализата и спектроскопије у подручју спектра блиском инфрацрвеном

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САЖЕТАК

Увод/Циљ Дијабетесна нефропатија води ка трајном оштећењу бубрежног ткива, док сам хемодијализни третман проузрокује осцилације у нивоу глукозе у крви. Неинвазивни мониторинг глукозе, кроз скенирање отпадног дијализата, пружио би значајне информације о нивоу глукозе у крви болесника.

Циљ студије је предикција концентрације глукозе у крви болесника на хемодијализи кроз спектроскопску карактеризацију отпадног дијализата у подручју спектра блиском инфрацрвеном (*NIR*).

Методе Узорци крви и отпадног дијализата узимани су од 15 болесника у 15. минуту хемодијализе. Узорци отпадног дијализата скенирани су у региону *NIR*, који се простирао од 900 до 1300 *nm*. Да би се применила вештачка неуронска мрежа, коришћена је функција *NFTOOL* програмског пакета *Matlab*. Испитивање и обука вештачке неуронске мреже изведени су коришћењем спектра *NIR* отпадне дијализне течности као улаза и концентрације глукозе у дијапазону 3–9 *mmol/l* као излаза.

Резултати Користећи вештачку неуронску мрежу, уочили смо значајну корелацију између спектра отпадног дијализата и концентрације 3–9 *mmol/l* глукозе у крви болесника.

Закључак Корелација од 93% између спектра *NIR* отпадног дијализата и концентрације глукозе показала је да се спектроскопија *NIR* може сматрати неинвазивном методом за поуздано праћење нивоа глукозе у крви код болесника на хемодијализи.

Кључне речи: хемодијализа; машинско учење; отпадни дијализат; спектроскопија *VIS-NIR*; индивидуализовани мониторинг

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Dislocation after primary unilateral total hip arthroplasty – hip geometry and risk factors (a matched cohort analysis)

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SUMMARY

Introduction/Objective The purpose of this study was to determine if patient-related factors, such as hospitalization length and preoperative use of walking aids, and geometrical factors, measured with antero-posterior radiographs of hip, affect the risk of hip dislocation after total hip arthroplasty.

Methods A total of 36 of 433 (8.31%) patients with hip dislocation were identified in the institutional registry during a two-year period. The data for patients with and without hip dislocation were matched and compared.

Results Hip dislocation more often occurred in patients who had used walking aids before the operation compared to the ones who had not ($p < 0.001$). Also, a difference in the number of hip dislocations was noticed between the patients who stayed longer in the hospital after THA ($p < 0.001$). The patients with higher acetabular inclination angle ($p < 0.005$) and height of greater trochanter ($p < 0.001$) on radiographs had been more prone to hip dislocation. In addition to this, the "safe-zone" was not identified in our study ($p > 0.005$).

Conclusion Several factors which influence hip dislocation were identified in this study: patient characteristics and radiograph characteristics. Both groups of factors require attention and monitoring in future studies.

Keywords: hip dislocation; biomechanics; total hip arthroplasty, complications; "safe zone"

INTRODUCTION

Total hip arthroplasty (THA) is an effective, common and costly operation [1]. It is considered the most successful procedure in orthopedic surgery since it relieves pain, increases mobility and quality of life, and provides a high level of patient survival [2]. Despite the efficacy of THA, hip dislocation (HD) represents a major problem after THA [2, 3]. The annual rate of HD after primary THA was reported to be 0.1–10%, while revision due to HD dislocation was reported to represent 9–26% of all revisions of primary THAs [3, 4, 5].

Multiple factors have been suggested to contribute to HD [3, 6, 7]. Operation-specific risk factors include the hospital volume, surgeon's experience, surgical approach, suboptimal positioning of the acetabular and femoral component, soft-tissue imbalance, etc. [5, 7]. As the procedure-specific factors, acetabular cup diameter, femoral head diameter, femoral neck length, head-to-cup ratio, procedure type, and the use of a liner were analyzed [8]. Although treatment outcome highly depends on the quality of surgical reconstruction of anatomical and biomechanical relations of the bone tissue [3], HD was noticed to occur even in the absence of procedure-specific mistakes. Thus, patient-specific risk factors for HD, including advanced age, high body mass index, comorbidities (especially psychiatric and neurologic diseases), low physical activity level, preoperative use of walking aids (PUWA), impaired compliance (failure to comply to permitted activities after surgery), and absence of exercise therapy, were suggested as important [7, 9, 10, 11]. According to the most recent studies, the length of stay (LOS) after THA has been shortened [4, 7, 8]. Out of many different patients' and providers' characteristics that determine LOS, comorbidity is the most documented one [12, 13]. It is also known that early mobilization results in the reduction of LOS and cost outcomes [14].

Despite the number of researches done, the risk factors of HD are not yet fully understood [1, 15]. Recent studies examine the influence of factors such as alcohol consumption, some diseases, and postoperative activity restrictions, but also the existence of the safe zone for cup

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position in HD occurrence [6]. The main limitation of the majority of published studies are the following: a small number of risk factors were analyzed, they failed to report the data on functional abilities of patients before and after the surgery, did not provide a description of procedures used in physical rehabilitation, did not perform long-term follow up of patients, etc.

The main aim of our study was to assess the link between patient-related factors and hip geometry related to the incidence of HD after THA.

METHODS

Patients

This clinical monocentric study was performed by prospectively gathering data on 433 patients that were subjected to THA between January 2016 and December 2017. Some patients were surgically operated due to nontraumatic indications, while others needed urgent surgery due to traumatic indications. Oral and written informed consent was obtained from all patients. The study was done in accordance with the institutional committee on ethics.

By analyzing hospital records, we identified 36 patients who experienced HD after THA. HD was identified as an episode that required closed or open reduction of a THA prosthesis. In cases of multiple HD, only the first occurrence was evaluated. Since radiographs of two HD patients were not adequate for analysis (there was no visible lesser trochanter and iliac crest), these two HD patients were excluded from the analysis, which resulted in a total of 34 HD patients. The control group consisted of 34 patients, operated on during the same period and under the same conditions, who did not experience HD after THA. The controls were matched to HD patients, using basic patient characteristics: age at the time of primary THA (± 3 years), sex, etiology responsible for THA (traumatology/non-traumatology), type of prosthesis (exact), comorbidities (Charlson Comorbidity Score), and physical activity level before the operation. The exclusion criteria were evidence of infections, malignant disease, instability, THA revision, other major joint arthroplasty or orthopedic surgery on the lower extremity one year before THA. Patients included in the study were followed-up for six months.

Surgical protocol

The patients were operated on by 13 surgeons, 10 of whom had 5–25 years of experience, and three of whom less than five years of experience. All the patients were operated on according to the protocol of the Clinical Center, which requires the surgery to be done under general anesthesia and posterior approach, without reconstruction of the external rotators. Out of 433 patients a total of 100 patients received cement type of prosthesis (patients older than 65); cement type prosthesis was received by seven HD patients and nine controls. Patients younger than 65 received a noncement (total n = 240, 17 HD patients, and 17 controls) and hybrid (total n = 93, 10 HD patients, and eight controls) type of prosthesis. The choice of prosthesis components and prosthesis size were at the discretion of the attending surgeon. Different designs (Implancast, DePuy, Zimmer, Stryker, Zimmer/Biomet) of cup/stem and femoral head sizes (28 or 32 mm) were used. The bearing surface for all prostheses was polyethylene on metal. In patients with a noncement and hybrid type of prosthesis, a head-on-polyethylene liner was used.

Postoperative protocol

During hospitalization, all the patients had physical therapy according to the standard protocol. The patients were verticalized immediately after the intervention, walked on crutches, with or without load-bearing on the leg that was operated on, depending on the type of THA and the surgeon's opinion. Physiotherapy took place daily, except on weekends. General postoperative restrictions for the first three months were used.

Patient and implant characteristics

Baseline patients' data included age at the time of primary THA, sex, THA side, comorbidities, physical activity level, and etiology (diagnosis responsible for THA). We used the Charlson index by defining the 19 comorbid conditions [16]. In addition to the Charlson score, individual comorbidities were included for separate analysis, consisting of diabetes mellitus, rheumatoid arthritis, peripheral vascular disease, neurologic disease, pharmacologically treated psychiatric disease and consummation of more than two units of alcohol daily. Physical activity level, according to Devane et al. [17], was quantified as the level 0–5.

Pre/postoperative data included analysis of mechanisms and time of dislocation, PUWA, LOS in hospital after surgery, and implementation of exercise therapy before and after THA. The patients were asked about any trauma or motions that led to the HD, if event represented the first or recurrent dislocation, how long ago the primary THA was performed, and whether they were subjected to physical and exercise therapy.

Operative notes were used to identify the operative side, surgeon, implant type, cup size, and femoral implant diameter.

Measurement of radiographic variables was performed using standard anteroposterior radiographs made immediately after THA. Measurements were performed by two independent authors, twice for each radiograph. The mean value of the four measurements was used for analysis. The reconstruction of the hip rotation center was performed by drawing a circle around the femoral head. Köhler line was drawn along the medial aspect of the ilium and ischium. First, a line through the base of the acetabular teardrop was drawn (Line 1). Then, a Köhler line was drawn from the lateral border of the sciatic notch to the medial border of the obturator foramen. Finally, a line was drawn through the center of the femoral head to the iliac crest (Line 2). The acetabular teardrop was used, as a reference since it

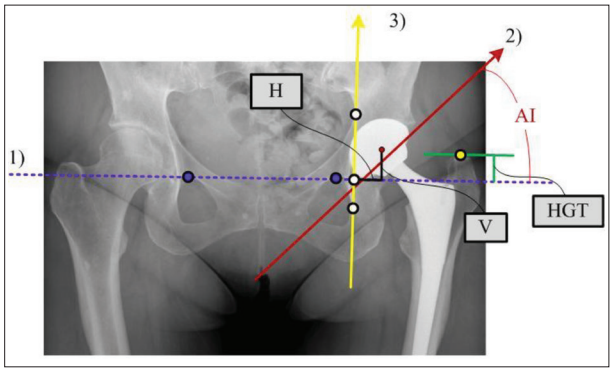

Figure 1. Representation of radiographic measurement parameters; Line 1 – horizontal; Line 2 – for AI determination; Line 3 – Köhler line; AI – acetabular inclination; HGT – height of greater trochanter; V – vertical offset; H – horizontal offset

represents an accurate method to measure distances [12, 15]. The lateral lip of teardrop indicates the exterior acetabular wall. Cup position was assessed according to the acetabular abduction angle (the angle between Line 1 and Line 2), vertical offset and the horizontal offset. Vertical offset was measured from the center of the femoral head to Line 1. The horizontal offset was measured from the center of the femoral head to the Köhler line (normal). The radiographic reconstruction of the abductor mechanism was measured using the height of the greater trochanter (HGT) as the distance between the Line 1 and the parallel line crossing the tip of the greater trochanter (Figure 1).

Statistics

The study data were analyzed by descriptive statistics and presented in tables. The mean value was used as a measure of central tendency and standard deviation as a measure of dispersion for continuous variables. The values of categorical variables were presented as rates or percentages. The normality of data distribution was tested by the Kolmogorov–Smirnov test. A χ^2 test was used to assess the difference in the distribution of categorical data between the HD group and the control group. Student's t-test or Mann–Whitney test was used to assess differences in mean values of interval data. Statistical analysis was performed in SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Out of 433 patients, 36 patients experienced HD, representing a rate of 8.31%. Thirty-three HD patients were stable after non-operative treatment, while three HD patients needed a revision. Out of 36 HD patients, two were excluded from the analysis, due to technical problems with radiographs.

HD patients and control patients did not significantly differ regarding their age, side that was operated on, THA etiology, Charlson comorbidity score, neither were individual comorbidities more frequent in HD patients. Also, physical activity levels were similar in HD and control patients.

Table 1. Basic patient data

Parameter	HD patients	Control patients	p
Age (X ± SD)	66.05 ± 10.22	64.79 ± 9.85	p > 0.05[a]
Sex (n)			
Male	10	10	p > 0.05[b]
Female	24	24	
THA side (n)			
Left	13	16	p > 0.05[b]
Right	21	18	
THA etiology			
Primary OA	17	20	p > 0.05[b]
Congenital hip disorder	1	2	
Rheumatologic disorders	0	1	
Trauma	12	8	
Avascular necrosis	3	1	
Metabolic bone disorders	1	1	
Other	0	1	
Charlson comorbidity score			
0	1	3	p > 0.05[b]
1	1	2	
2	4	6	
3	7	9	
4	7	7	
5	5	2	
6	3	3	
7	4	1	
8	2	1	
Individual comorbidities (n)			
Neurological diseases	2	1	p > 0.05[b]
Diabetes mellitus	3	4	
Psychiatric diseases	6	1	
Vascular diseases	3	1	
Rheumatoid arthritis	0	2	
Alcohol consumption	1	0	
Physical activity level			
0	0	0	p > 0.05[b]
1	0	0	
2	6	7	
3	16	13	
4	11	11	
5	1	3	

THA – total hip arthroplasty; HD – hip dislocation;
[a]Mann–Whitney test;
[b]χ^2 test

There was no statistically significant difference in the occurrence of HD after THA if the patient was operated on by the surgeon with less or more experience ($p > 0.05$, χ^2 test). There was a similar number of patients receiving pre/postoperative rehabilitation treatment in both groups, but PUWA was more frequently used in HD patients compared to control patients. HD patients also spent significantly more time in hospital after THA. Time of dislocation in the HD group ranged 3–3300 days after THA (median 282.50), and the most frequent mechanism was inappropriate movement. Late dislocations (> 90 days after THA) were more frequent than the early ones.

HD patients and control patients did not significantly differ in acetabular shell size, cup anteversion angle, the

Table 2. Pre/post total hip arthroplasty-related data.

Parameter	HD patients	Control patients	p
PUWA (n)			
Yes	19	4	p < 0.001[b]
No	15	30	
LOS (days)	11.00 ± 2.71	8.82 ± 1.66	p < 0.001[a]
Rehabilitation (n)			
Preoperative (Yes/No)	17/17	14/20	p > 0.05[b]
Early postoperative (Yes/No)	34/0	34/0	
Post THA (Yes/No)	11/23	14/20	
HD mechanism			
Falls	5	/	p < 0.001[b]
Inappropriate movement	11		
Sitting	3		
Bending	5		
Squatting	1		
Unknown	9		
Time of dislocation (n)			
Early/Late	14/20	/	p < 0.001[b]

THA – total hip arthroplasty; HD – hip dislocation; PUWA – preoperative use of walking aids; LOS – length of stay;
[a]Mann–Whitney test;
[b]χ^2 test

Table 3. Implant and radiographic data

Parameter	HD patients	Control patients	p
Acetabular shell size (mm)	51.08 ± 13.90	53.61 ± 3.77	p > 0.05[a]
Femoral head size (mm)			
28 mm	18	13	p > 0.05[b]
32 mm	16	21	
Acetabular inclination (degrees)	47.52 ± 6.07	45.18 ± 2.98	p < 0.05[c]
Horizontal offset(mm)	31.00 ± 4.85	29.22 ± 3.07	p > 0.05[a]
Vertical offset (mm)	22.50 ± 6.11	23.06 ± 5.69	p > 0.05[a]
Height of greater trochanter (mm)	2.27 ± 2.88	0.46 ± 1.30	p < 0.01[a]
Cup position			
Inside safe zone[d]	3	2	p > 0.05[b]
Outside safe zone[d]	21	32	
Abductor mechanism			
Inside safe zone[d]	2	1	p > 0.05[b]
Outside safe zone[d]	32	33	

HD – hip dislocation;
[a]Mann–Whitney test;
[b]χ^2 test;
[c]Student's t-test;
[d]safe zone defined as 40 ± 10 abduction, 15 ± 10 anteversion for cup for abductor mechanism

horizontal and vertical offset of a cup, nor the frequency of different femoral head sizes, cup position zone, and abductor mechanism zone. However, HD patients had significantly higher acetabular inclination angle and height of greater trochanter when compared to control patients.

DISCUSSION

HD remains the major complication after THA. While multiple reasons may be contributing factors leading to dislocation, precise identification of the exact reason is of major importance. As with any multifactorial problem, the prediction of postoperative outcomes is difficult. The results of our study have shown that LOS, PUWA and acetabular inclination and HGT are associated with the occurrence of HD.

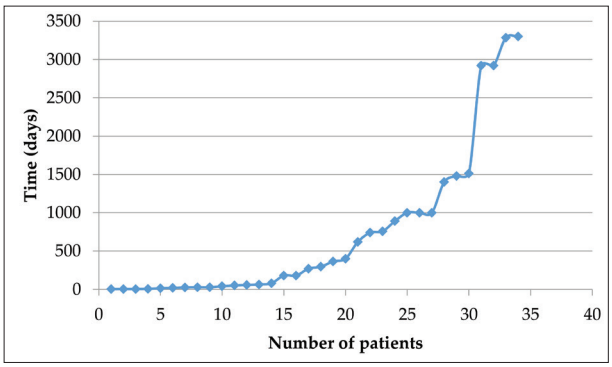

Figure 2. Time of occurrence of hip dislocation

Understanding the factors associated with the occurrence of HD can help plan the operation, preventing the occurrence of HD and reducing treatment costs [3, 4, 10, 18, 19]. Since the Republic of Serbia does not have a national register of patients with THA, we used systematically collected data from our medical center.

In most of the previous studies, it was shown that many factors are associated with HD, but these studies predominantly investigated surgical factors, which were not in the focus of our research [18]. In our medical center, all surgeons use a posterior approach, the same type of prosthesis, and the same operating technique.

The main purpose of this study was to assess the patient-related factors and radiological factors on HD occurrence after THA.

HD is a significant problem in clinical practice [3, 4, 19]. Restoration of the native anatomy plays a crucial role in preventing instability [6]. Malposition of the acetabular component is associated with the occurrence of many complications, not only HD [20]. Some authors still accept some kind of "safe zone" for prosthesis placement [1, 4, 10], while others question whether it exists [6, 15].

Cup position with an inclination/abduction of 40° ± 10° and an anteversion of 10–20°, the so-called "safe zone" to avoid HD, is internationally considered desirable and used in clinical practice. However, experience showed that components positioned in this zone can and do dislocate [4]. This was confirmed by HD occurrence even after the use of computer-assisted surgery during THA [20]. As mentioned before, some authors argue that the "safe zone" does not exist or that it is different for each patient [6, 12]. Also, some authors claim that the "safe zone" depends on the operative approach or some other factor [20].

Hip geometrical parameters have been suggested as important factors in the evaluation of the risk of HD [3, 4, 10, 19]. Our results show that patients who experienced HD had the higher acetabular inclination and lower height of greater trochanter. Previous studies have shown that the height of the great trochanter depends on the soft tissue reparation and thigh muscle strength [1, 20]. Also, cup

position was not significantly different between study groups, which aligned with findings of Esposito et al. [6]. This indicates that muscle strength is of great importance in the prevention of HD [14].

There is no consensus in literature data about optimal acetabular orientation since different referent systems, surgical techniques, and measurement techniques were used. The malposition of the acetabulum can lead to numerous complications among which is HD. This is explained by bigger bearing surface and instability [20].

The number of all observed HDs (n = 34, 8.31%) in our study is on the upper limit described in the literature (0.3–10%) [1, 3, 4]. If we follow only operatively treated HDs (three patients) frequency would be 0.69%. There are several factors such as the posterior operative approach, middle hospital volume, lack of surgeon experience, which could explain this high incidence of HD [4, 13]. Also, we included a wide range of patients in our study, even the patients with an increased risk for HD – older patients with cement prosthesis, posttraumatic THA, psychiatric patients, and patients with neuromuscular impairment. Older age is associated with a lack of coordination, senses and muscle weakness, comorbidity, poor compliance, and preference to falling. Abovementioned characteristics of older patients increase the risk of HD.

Data that come from national registers or multicentric studies took patients with "ideal" characteristics such as large femoral head, non-cement prosthesis, and numerous exclusion criteria [3, 19]. The advantage of our study was that we did not have a loss of patients because each patient treated in our hospital was adequately followed as there is no other hospital where HD could be treated.

Another potential risk factor for HD is the so-called early mobilization regimen of physical therapy [21]. Every patient in our medical center is subsided to early mobilization, so we were not able to evaluate it as a risk factor.

Surgeon experience and volume were not identified as significant factors for the occurrence of HD. All surgeons in our medical center had low patient volume.

Previous research showed that physiotherapy after THA enhanced postoperative recovery by promoting faster rehabilitation and improving functional outcomes [11, 21, 22]. It is argued that even though intensity and frequency of the ideal rehabilitation protocol are unknown, early multidisciplinary rehabilitation improves outcomes [11]. However, our results did not show that physiotherapy is a protective factor. The reason could be that physiotherapy is mandatory in our medical center for all patients after THA. Also, the number of patients included in our study could be insufficient to show the significance of physiotherapy.

The number of patients who suffered from HD is 14 in the first 90 days of surgery, and 20 patients after 90 days. Similar results were obtained in the study of Kunutsor et al. [3] where half of all HDs occurred in the first three months postoperatively.

LOS is an important component of the recovery and indicator of the overall cost after THA. Since the implementation of fast track surgery, there is a tendency to reduce the LOS in hospital to reduce costs, reduce the number and seriousness of complications, and increase the number of available hospital beds [13]. Every hospitalization longer than four days or 10 days is considered prolonged hospitalization [9, 22]. Shorter LOS is associated with better motivation and satisfaction of patients during recovery [9].

Some factors associated with longer LOS (age, sex, comorbidity, economic status, PUWA) are better documented than others (surgeon volume, infections, general anesthesia). High-quality studies should provide more evidence for the relationship between LOS and mentioned factors [21, 22].

Jørgensen et al. [7] showed that longer LOS could increase the risk of complications but not the risk of HD. On the other hand, shorter LOS does not mean more HDs [9], which is contradictory with findings of Mauerhan et al. [23]. In our study, LOS was 6–14 days, which is in line with other studies where a conventional surgical track regimen was used. Different treatment concept assumes a postoperative early mobilization program. Larger value of BMI is associated with longer LOS and increasing costs [24].

We showed that HD patients spent more time in hospital after THA and more frequently needed PUWA, which reflects their lower pre/post physical capabilities.

PUWA is associated with the occurrence of medical and non-medical complications [25]. PUWA is a sign of loss of hip muscle strength and in most cases is associated with older age and/or comorbidity [11]. Age over 70 years, female sex, depression, and BMI over 35 are highly associated with PUWA [22]. More research is needed to examine the impact of specific comorbidities on PUWA [25].

Results of our study showed that PUWA is a risk factor for HD which is aligned with the results of the study by Jørgensen et al. [25]. The impact of PUWA must be additionally confirmed in new studies.

There are several limitations to our study. The most significant one is that we investigated a three-dimensional problem by using two-dimensional X-ray images for measuring acetabular inclination. However, femoral anteversion was not measured. Also, we did not have access to the software made for the use of measuring these parameters. All investigated parameters were measured manually. The number of patients included in our study is relatively small and the follow-up period is short, compared to other studies. Limitations of our study also include loss of some radiographic data, the lack of detailed registration of patient compliance to restrictions and missing clinical information including the loss of BMI data, absence of detailed functional results of the THA according to a clinical scale.

CONSLUSION

HD is a serious complication that may be attributed to the multiple factors. Having in mind results presented in this study, we suggest implementation of PUWA, LOS, and hip geometry monitoring in addition to other well-known risk factors.

Conflict of interest: None declared.

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Дислокација после уградње примарне унилатералне тоталне ендопротезе кука – геометрија кука и фактори ризика (упарена кохортна студија)

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САЖЕТАК

Увод/Циљ Циљ истраживања је да утврди на који начин фактори као што су дужина постоперативне хоспитализације, употреба помагала за ходање пре операције као и геометријски фактори који су мерени на антеро-постериорном радиографском снимку утичу на ризик од настанка дислокације кука после тоталне ендопротезе кука.

Методе Коришћењем институционалног регистра током двогодишњег периода идентификовано је 36 болесника (8,31%) са дислокацијом кука, од укупно 433 болесника са тоталном ендопротезом кука.

Резултати Добијени подаци болесника са дислокацијом и без дисколације су упоређивани и анализирани. Дислокација кука била је чешћа код болесника који су пре операције користили помагала за кретање у односу на оне који нису ($p < 0,001$). Значајна разлика је регистрована код болесника код којих је хоспитализација после операције трајала дуже ($p < 0,001$). Болесници са већим углом инклинације ацетабулума ($p < 0,005$) и вишим великим трохантером ($p < 0,001$) на радиографским снимцима су чешће имали дислокацију. Поред тога, „сигурна зона" у нашој студији није идентификована.

Закључак У овом истраживању индентификовни су фактори који су у вези са дислокацијом кука као што су дужина постоперативне хоспитализације и употреба помагала за ходање пре операције. Такође, идентификовани су и радиографски фактори, који заслужују даљу пажњу и праћење у будућим истраживањима

Кључне речи: дислокација кука; биомеханика; тотална артропластика кука, компликације; „сигурна зона"

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Is radiofrequency use in arthroscopic treatment of isolated medial meniscus horizontal cleavage tears more effective than mechanical debridement in young adults?

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SUMMARY

Introduction/Objective Arthroscopic mechanical hand tools, motorized shavers, and bipolar radiofrequency are used in arthroscopic partial meniscectomy.
The aim of this study is to evaluate efficacy of radiofrequency on early clinical outcomes in patients who underwent arthroscopic partial meniscectomy with horizontal cleavage tear and without additional intraarticular knee pathology.

Methods A total of 37 patients complied with the study criteria. Patients were divided into two groups according to usage of bipolar radiofrequency. Patients were evaluated by using visual analog scale (VAS) and Tegner Lysholm knee scores at the end of the first year follow-up.

Results Twenty-two patients comprised the shaver-using group. Preoperative mean VAS score was 7.9 ± 0.8, and the Tegner Lysholm knee score was 49.6 ± 9.6. Fifteen patients comprised the bipolar radiofrequency-using group. Preoperative VAS score was 7.8 ± 0.9, and the Tegner Lysholm knee score was 52.2 ± 10.7. The mean VAS score was 1.2 ± 0.9, and the mean Tegner Lysholm knee score was 89.5 ± 8.1 in shaver used group at last follow-up. At the last postoperative follow-up, the mean VAS score was 1.1 ± 1, and the Tegner Lysholm knee score was 88.8 ± 7.3 in the bipolar radiofrequency-using group. No statistically significant differences between the VAS and Tegner Lysholm knee scores of the preoperative and postoperative controls of the two groups were observed (p≥0.05).

Conclusion Radiofrequency use has no effect on early clinical outcomes in the arthroscopic treatment of isolated medial meniscus posterior horn horizontal cleavage tears; we do not recommend its use.

Keywords: radiofrequency; horizontal cleavage tear; meniscus

INTRODUCTION

Horizontal cleavage tears in young people were first described in 1993 by Biedert et al. [1]. Horizontal cleavage tears have a traumatic or non-traumatic etiology during degeneration caused by disruption of the load distribution in the knee joint. Horizontal cleavage tears extend to the inferior surface of the meniscus [2].

Degenerative meniscus tears usually involve horizontal cleavage tears and are more common in middle-aged patients. The prevalence of horizontal cleavage tears increases with age [3]. A mechanical relationship between meniscus degeneration and knee osteoarthritis is believed to exist, but no consensus on the relationship between the structures of the cartilage and meniscus and the sequence of degeneration is yet available. Conservative treatments, arthroscopic meniscus sutures, and arthroscopic partial meniscectomy are performed in the treatment of horizontal cleavage tears [4].

Arthroscopic mechanical hand tools, motorized shavers, and bipolar radiofrequency are used in arthroscopic partial meniscectomy. The use of radiofrequency can cause cell death due to thermal effects on cartilage tissue. Allen et al. [5] found that cell death rates do not differ between debridement with punch and mechanical shaving and meniscus debridement using bipolar radiofrequency. The authors thus stated that bipolar radiofrequency does not damage the cartilage of joint surfaces and provides a smooth contour on the meniscus surface [5].

The aim of this study is to evaluate retrospectively the efficacy of radiofrequency on early clinical outcomes in patients who underwent arthroscopic partial meniscectomy with horizontal cleavage tear and without additional intra articular knee pathology.

METHODS

This retrospective study was approved by the ethics board of Bahçeşehir University, Faculty of Medicine, (No. 2019-18/01) and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all patients included in the study.

A total of 218 patients who underwent knee arthroscopy in our clinic in 2018 were evaluated retrospectively in our study. The inclusion criteria were ages 20–50, detection of tears in

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the posterior horn of the medial meniscus on preoperative magnetic resonance imaging, and detection of only horizontal cleavage tears in the posterior horn of the medial meniscus without any other intraarticular knee pathology during arthroscopy. Patients with visual analog scale (VAS) and Tegner Lysholm knee scores in their preoperative and postoperative follow-up files were included in the study. Patients who were under 20 and over 50 years of age, with inflammatory arthropathy, who had cartilage problems in the medial, lateral, or patellar femoral joints, who had lateral meniscus tear or anterior cruciate ligament injuries in addition to the horizontal cleavage tear in the medial meniscus, and those with trauma history were excluded from the study.

Surgical technique

Prophylaxis of the patients before surgery was performed with 1 g of cefazolin sodium. A tourniquet was applied to the extremity under general or spinal anesthesia. An anterolateral portal was created in all surgeries, and an anteromedial portal was created after diagnostic arthroscopy. First, the patellofemoral joint, lateral, and medial gutters were evaluated. The medial compartment medial meniscus, anterior cruciate ligament, lateral compartment, and lateral meniscus were then evaluated. Partial meniscectomy using appropriate angled punches was performed for patients who had horizontal cleavage tears in the posterior horn of the medial meniscus. During partial meniscectomy, the upper and lower flaps were excised by preserving the outer 50% of the meniscus. Meniscus contours were smoothed by using a shaver in some patients and bipolar radiofrequency in some patients. After the tourniquet was deflated, the portals were closed.

Postoperative follow-up and patient data collection

All patients underwent a postoperative physical therapy program and were evaluated using VAS and Tegner Lysholm knee scores at the end of the first year of follow-up [6]. The surgical and follow-up files of patients who underwent arthroscopy with a follow-up period of over one year were evaluated retrospectively. A total of 37 patients complied with the study criteria. When the patients' operations were evaluated, we determined that bipolar radiofrequency was not used during the arthroscopic partial meniscectomy of 22 patients. Bipolar radiofrequency was used during the arthroscopic partial meniscectomy of 15 patients. Patients were divided into two groups according to bipolar radiofrequency use.

Statistical analysis

The suitability of the data to a normal distribution was tested. Because the data were not normally distributed, pre- and postoperative VAS and Tegner Lysholm knee scores were analyzed by using the Wilcoxon test, which is a non-parametric version of the paired t-test. Differences in VAS and Tegner Lysholm knee scores between the bipolar radiofrequency-using and -non-using groups were also evaluated by the Mann–Whitney U test. A p value of < 0.05 was considered statistically significant at the 95% confidence interval.

RESULTS

Of the 37 patients who met the study criteria, 25 were male and 12 were female. The mean age of the patients was 38.6 ± 5.1 years. Patients included in our study mostly consisted of heavy workers. The lifestyle in Turkey was the main reason for younger average age for horizontal cleavage tears. Of the 22 patients without bipolar radiofrequency use during arthroscopic partial meniscectomy, 16 were male and six were female. The mean follow-up period was 13 ± 2.1 months in this group. The preoperative mean VAS score was 7.9 ± 0.8, and the Tegner Lysholm knee score was 49.6 ± 9.6. Ten of the 15 patients in the bipolar radiofrequency-using group were male; the rest were female. The mean follow-up period was 12 ± 9.4 months. In this group, the preoperative VAS score was 7.8 ± 0.9, and the Tegner Lysholm knee score was 52.2 ± 10.7. The mean VAS score was 1.2 ± 0.9, and the mean Tegner Lysholm knee score was 89.5 ± 8.1 in group which radiofrequency was not used at the last follow-up. At the last postoperative follow-up, the mean VAS score was 1.1 ± 1, and the Tegner Lysholm knee score was 88.8 ± 7.3 in the bipolar radiofrequency-using group (Table 1). Statistically significant differences in the VAS and Tegner Lysholm knee scores of the preoperative and postoperative final controls of both groups ($p \leq 0.05$) were observed. No statistically significant differences in the VAS and Tegner Lysholm knee scores of the preoperative and postoperative controls of the two groups ($p \geq 0.05$) were found.

Table 1. Preoperative and postoperative clinical scores of groups

Time	Group with shaver	Group with radiofrequency
Pre-op VAS score	7.9 ± 0.8	7.8 ± 0.9
Post-op VAS score	1.2 ± 0.9	1.1 ± 1
Pre-op Tegner Lysholm scores	49.6 ± 9.6	52.2 ± 10.7
Post-op Tegner Lysholm scores	89.5 ± 8.1	88.8 ± 7.3

VAS – visual analogue scale

DISCUSSION

Complex meniscal tears and cartilage defects are common among patients with early and advanced knee osteoarthritis. Pain and restriction of range of motion are also higher in this group of patients than in groups without knee osteoarthritis [7]. Major meniscus problems are more common in patients who need knee replacement than in those who do not need knee replacement [8, 9]. In particular, knees with macerations in the meniscus body and posteromedial horn are more in need of knee arthroplasty than control knees [8]. Antony et al. [10] found that the meniscal changes, such as meniscus maceration and meniscus extrusion, are more likely to cause structural changes than meniscus signal increases and meniscus tears. The authors hence determined that meniscal maceration has

an effect on knee pain and knee osteoarthritis [10]. Horizontal cleavage tears are meniscus tears extending from the avascular zone to the vascular zone and considered degenerative meniscus tears [11]. Previous studies suggested that the earliest anatomic finding of knee osteoarthritis is a degenerative tear of the medial meniscus posterior horn. The aim of the present study is to exclude patients with chondral lesions and compare the treatment results of isolated medial meniscus horizontal cleavage tears by using bipolar radiofrequency and shaver.

Although patients with traumatic meniscus tears were not included in our study, Kim et al. [12] divided 40-year-old patients with horizontal cleavage tears into two groups according to the etiology of their meniscus tear as traumatic or degenerative meniscus tears. IKDC scores increased from 58.1 to 84.6 in the traumatic group and from 59.1 to 85.1 in the non-traumatic group. Lysholm scores increased from 61.1 to 85.5 in the traumatic group and from 62.2 to 86.1 in the non-traumatic group. No statistically significant difference was found between the two groups [12]. According to our results, a statistically significant increase in Lysholm scores exists between the preoperative and postoperative final controls.

Partial meniscectomy is recommended for the treatment of degenerative horizontal cleavage tears because these tears are located in the avascular region and, therefore, have lower healing potential compared with acute traumatic tears [13]. Koh et al. [14] found a decrease in contact area and increase in pressure on the medial compartment when they removed the inferior portion of horizontal cleavage tears in their study on cadaveric knees. The authors of that study found no further reductions in contact area or increases in pressure in medial femoral condyles with additional excision of the superior part of the horizontal cleavage tear [14]. Uquillas et al. [15] found that resection of the upper or lower flap in horizontal cleavage tears or resection of both flaps does not change the pressure on the contact surface. The authors thus recommended the preservation of the outer 50% of the medial meniscus in horizontal cleavage tears in the posterior horn of the medial meniscus [15]. In our study, we attempted to preserve 50% of the peripheral portion of the meniscus of patients who underwent partial meniscectomy, and we excised both flaps.

Another approach in the treatment of horizontal cleavage tears is meniscus repair. Arno et al. [16] found a 13% increase in contact pressure and 6% decrease in contact area as a result of horizontal cleavage tears in their cadaver study. The authors thus stated that horizontal cleavage tears may cause cartilage degeneration by causing few but statistically significant changes in tibiofemoral contact mechanics [16]. Beamer et al. [17] conducted a study on cadaver knees and determined that contact area and pressure on the contact area are close to normal values after the repair of horizontal cleavage tears with sutures. Moreover, the contact area decreased and the pressure on the contact area increased in knees treated with subtotal or partial menisectomy [17]. Billières et al. [18] performed partial meniscectomy and open meniscus repair in 14 patients with degenerative horizontal cleavage tear and found a mean International Knee Documentation Committee score of 86.1 ± 10.9 after 8.5 years of follow-up. The authors also found the mean Knee Injury and Osteoarthritis Outcome Scores of 91.4 ± 7.5 for pain, 91.4 ± 10.2 for symptoms, 97.1 ± 4 for daily activity, 84.4 ± 20.7 for sports, and 84 ± 14.2 for the quality of life. The authors thus reported that repairing complex horizontal cleavage tears in young patients yields good subjective and objective results [18]. Despite these findings, however, negative results of horizontal cleavage tears after meniscus repair have been published. Biedert et al. [4], for example, compared four groups, including those conservatively treated, treated by meniscus suture, treated by meniscus suture using fibrin glue, and treated by partial meniscectomy. The scholars reported that the best short-term clinical results are obtained in the group treated by partial meniscectomy [4]. Shanmugaraj et al. [19] found that repair of horizontal cleavage tears has a higher complication rate than repair with partial meniscectomy. Since a meniscus repair group was not present in our study, our findings are somewhat limited.

Spahn et al. [20] performed arthroscopic debridement on patients with grade 3 chondropathy and degenerative tears of the medial meniscus. Here, cartilage debridement was performed by using radiofrequency in one group and shaving in another group. The authors reported that the clinical results of the radiofrequency group are better than those of the shaver group after one year of follow-up [20]. Spahn et al. [21] reported that the results of debridement with radiofrequency are better than those of mechanical debridement after four years.

Figueroa et al. [22] investigated thermal effects on the meniscus using bipolar radiofrequency and found cellular changes in deeper tissues with bipolar radiofrequency in elderly patients; the authors thus recommended that precautions be taken during the use of bipolar radiofrequency in elderly patients during meniscectomy and advocated the use of low intensities. Allen et al. [5] found no difference in cell death rate between meniscal debridement with bipolar radiofrequency and debridement with motorized shaver and punches. It was concluded that radiofrequency does not damage the joint surfaces under the meniscus.

Grana et al. [23] compared mechanical meniscectomy with meniscectomy using radiofrequency and found that cracks and clefts are formed on surfaces on which meniscectomy was performed mechanically; by comparison, the surfaces of menisci subjected to meniscectomy with radiofrequency were flatter and more homogeneous. The author further found that meniscectomy with radiofrequency offers more resistance than mechanical meniscectomy in static tests [23].

Vangsness et al. [24] studied the effects of monopolar and bipolar radiofrequency devices on tissue during meniscectomy. No difference was detected between the two systems, and the use of radiofrequency factors, such as geometric shape of the probe, was reported to have an effect on the depth of thermal effect [24]. Studies showing the advantages of radiofrequency use and a low risk of osteonecrosis after use have been published. However, according to our findings, the results of partial meniscectomy using

radiofrequency and partial meniscectomy using shaver and punch are not significantly different.

CONCLUSION

We believe that radiofrequency use has no superior effect than mechanical debridement with motorized shaver and punches on early clinical outcomes in the arthroscopic treatment of isolated medial meniscus posterior horn horizontal cleavage tears in young adults. Usage of radiofrequency is high cost. Thus, we do not recommend its use.

Conflict of interest: None declared.

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Да ли је употреба радиофреквенције у артроскопском лечењу изолованог хоризонталног расцепа медијалног менискуса делотворнија од механичког прилагођавања код младих одраслих особа?

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САЖЕТАК

Увод/Циљ У делимичној артроскопској менискектомији користе се артроскопски механички ручни алати, моторизовани бријачи и биполарна радиофреквенција.

Циљ ове студије је да се процени ефикасност радиофреквенције на раним клиничким исходима код болесника који су били подвргнути делимичној артроскопској менискектомији са хоризонталним расцепом и без додатне интраартикуларне патологије колена.

Методе Укупно 37 болесника испунило је критеријуме студије. Болесници су подељени у две групе према коришћењу биполарне радиофреквенције. Оцењени су помоћу визуелне аналогне скале (ВАС) и скора *Tegner Lysholm knee* после годину дана праћења.

Резултати Двадесет два болесника су користила шејвер. Преоперативни скор ВАС-а је био 7,9 ± 0,8, а скор *Tegner Lysholm knee* 49,6 ± 9,6. Петнаест болесника чинило је биполарну групу која користи радиофреквенцију. Преоперативни резултат ВАС-а био је 7,8 ± 0,9, а скор *Tegner Lysholm knee* 52,2 ± 10,7. Просечан скор ВАС-а је био 1,2 ± 0,9, а средњи скор *Tegner Lysholm knee* 89,5 ± 8,1 у групи која је користила шејвер приликом последње контроле. Током последње контроле средња вредност ВАС-а била је 1,1 ± 1, а скор *Tegner Lysholm knee* је био 88,8 ± 7,3 у групи која је користила биполарну радиофреквенцију. Нису примећене статистички значајне разлике између скорова ВАС-а и *Tegner Lysholm knee* преоперативне и постоперативне контроле две групе ($p \geq 0,05$).

Закључак Коришћење радиофреквенције нема ефекта на рани клинички исход у артроскопском лечењу изолованог хоризонталног расцепа медијалног менискуса; не препоручујемо његову употребу.

Кључне речи: радиофреквенција; хоризонтални расцеп; менискус

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Relationship between optic nerve head topography and nerve fiber layer thickness with central corneal thickness in patients with primary open-angle glaucoma

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SUMMARY

Introduction/Objective In patients with primary open-angle glaucoma (POAG) we explored the relationship between the optic nerve head (ONH) topography parameters and the retinal nerve fiber layer (RNFL) thickness with the central corneal thickness (CCT).

Methods This retrospective study included 97 patients (97 eyes) with primary open-angle glaucoma. Patients were divided into a thin CCT < 540 μm (45 eyes) and a thick CCT ≥ 540 μm (52 eyes) group, using ultrasonic pachymeter. Topographic measurements of the ONH parameters and RNFL thickness was performed using optical coherence tomography (OCT). The outcomes were compared with the thin and thick CCT and correlated with the thin CCT of the subjects.

Results There were significantly lower mean intraocular pressure ($p < 0.0001$) and CCT ($p < 0.0001$) in patients with thin CCT compared to patients with thick CCT. Statistically significant differences of ONH parameters were found in thin cornea group compared to thick cornea group in: cup/disc area ratio ($p < 0.03$), vertical cup/disc ratio ($p < 0.01$) and rim volume ($p < 0.01$). Statistically significant differences of RNFL thickness were found in thin cornea group compared to thick cornea group in: average ($p < 0.001$), superior ($p < 0.03$), inferior ($p < 0.03$) and nasal ($p < 0.01$). Significant positive correlation was found between thin CCT and OCT parameters in: optic disc area ($r = 0.429$, $p = 0.003$), cup/disc area ratio ($r = 0.287$, $p = 0.05$), horizontal cup/disc ratio ($r = 0.472$, $p < 0.001$), vertical cup/disc ratio ($r = 0.578$, $p < 0.001$), average RNFL ($r = 0.796$, $p < 0.001$), superior RNFL ($r = 0.665$, $p < 0.001$), inferior RNFL ($r = 0.650$, $p < 0.001$), nasal RNFL ($r = 0.611$, $p < 0.001$) and temporal RNFL thickness ($r = 0.601$, $p < 0.001$).

Conclusion POAG patients with thin cornea will probably develop larger glaucoma changes than those with a thicker cornea. Ultrasonic pachymetry measurements of CCT and OCT analysis of ONH topography parameters and RNFL thickness provide significant information in early diagnosis and monitoring progression of POAG.

Keywords: intraocular pressure; ultrasonic pachymetry; optical coherence tomography

INTRODUCTION

Primary open angle glaucoma (POAG) is the disorder of the structural and functional changes of the optic nerve [1].

Glaucoma changes of the optic nerve may manifest as a morphological damage in the optic nerve head (ONH) as well as a decrease in the thickness of the retinal nerve fiber layer (RNFL) [2].

Optical coherence tomography (OCT) is a method usually used for evaluation of the structural glaucoma damage [3, 4]. OCT is non-contact and high-resolution device which provides a cross-sectional image, good for quantitative evaluation of the ONH and RNFL [5]. It is a repeatable time-saving procedure.

Central corneal thickness (CCT) is a risk factor for the development of POAG and a predictive factor for conversion ocular hypertension (OHT) to POAG [1]. It has been reported that thick cornea provides falsely elevated intraocular pressure (IOP), which may cause a false POAG diagnosis, whereas thin cornea provides an opposite result, which hides the risk of developing POAG [6]. Herendon et al. [7] reported that CCT was an important parameter of glaucoma ONH structural change. Hewitt et al. [8] also found that, in glaucoma eyes, thin CCT was related to increased vertical cup/disc ratio (VCDR).

The aim of this study was to determine whether thin CCT is associated with specific ONH topography parameters and RNFL thickness, measured by OCT in POAG patients.

METHODS

The is a retrospective study on documented 97 patients (97 eyes) with POAG at the Family Čivčić Ophthalmology practice in Belgrade.

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The research was done in accordance with the Helsinki Declaration and with the approval of the local Committee on Ethics.

Exclusion criteria were: myopia ≥ -6 D, secondary glaucoma, POAG advanced glaucoma stage, drusen of the optic nerve head and other anomalies of the optic nerve head, other ocular diseases, history of previous ocular surgeries and laser treatments, trauma, systemic comorbidities that may affect the visual field, patients with unreliable visual field (defined as false-negative errors > 33%, false-positive errors > 33%, and fixation losses > 20%), mean deviance (MD) ≥ -10 dB.

In all participants we examined best corrected visual acuity (BCVA), slit-lamp biomicroscopy, IOP measurement using Goldmann applanation tonometry, gonioscopy, a dilated fundus evaluation using indirect ophthalmoscopy with 90 D lens, and a 24-2 threshold test using a standard automated perimetry AP-1000 Tomey (Tomey, Nagoya, Japan). In addition, CCT was measured with ultrasonic pachymeter SP-100 Tomey (Tomey). ONH analysis (disc, cup and rim area, cup and rim volume, cup/disc (C/D) ratio, horizontal and vertical C/D ratio) with RNFL measurements (average and four quadrants RNFL thickness), was performed using spectral domain OCT (SOCT Copernicus Plus, Optopol Technology, Zawiercie, Poland).

POAG patients were classified into two groups according to their median CCT: thin CCT < 540 μm (45 eyes) and thick CCT ≥ 540 μm (52 eyes).

Demographic and clinical characteristics and OCT parameters were compared with the two groups according to the CCT value, using unpaired t-test (Microsoft Excel, Office 2010, Microsoft Corporation, Redmond, WA, USA). Pearson's correlation coefficients (r) were calculated to assess the associations between thin CCT and optic disc morphological parameters. Statistical analysis of Pearson 's correlation coefficients were performed by JASP version 0.12.2 (Jeffrey's Amazing Statistics Program, Amsterdam, the Netherlands). The significance level was set at p value of < 0.05.

RESULTS

This study included 97 eyes of 97 patients with medically controlled POAG. Of these, 39 (40.21%) patients were male and 58 (59.79%) patients were female. The average age of the examined population was 57.18 ± 13.05 (range 26–78) years. Demographic and clinical characteristics of patients with POAG were compared with the two groups according to the CCT value (Table 1). There was statistically significant difference in the mean IOP and CCT between the groups (p < 0.0001). IOP with the prescribed therapy was significantly higher in patients with thick CCT compared to patients with thin CCT (17.92 ± 2.40 mmHg *vs*. 15.62 ± 2.39 mmHg, p < 0.0001). CCT was significantly higher in patients with thick CCT compared to patients with thin CCT (569.65 ± 22.06 μm *vs*. 512.44 ± 20.39 μm, p < 0.0001). We found no statistically significant difference between the groups in terms of age, gender, and MD (p = 0.053, p = 0.65, p = 0.007).

Table 2 shows a comparison of ONH parameters obtained by OCT between two studied groups. There were statistically significant differences between thin CCT and thick CCT in these stereometric parameters: cup/disc area ratio (0.48 ± 0.15 *vs*. 0.42 ± 0.11, p < 0.03), VCDR (0.71 ± 0.12 *vs*. 0.69 ± 0.10, p < 0.01) and rim volume (0.12 ± 0.06 mm^3 *vs*. 0.15 ± 0.05 mm^3, p < 0.01). ONH parameters showed that cup/disc area ratio and VCDR were significantly larger and rim volume significantly smaller in POAG patients with thin CCT compared to patients with thick CCT.

The average and quadrant RNFL thickness were compared between the thin CCT and thick CCT. Statistically significant differences were found in thin cornea group compared to thick cornea group in: average (102.88 ± 11.04 μm *vs*. 110.32 ± 10.83 μm, p < 0.001), superior (118.42 ± 16.76 μm *vs*. 125.57 ± 15.82 μm, p < 0.03), inferior (118.44 ± 19.38 μm *vs*. 126.59 ± 16.93 μm, p < 0.03) and nasal (78.33 ± 12.39 μm *vs*. 84.15 ± 11.16 μm, p < 0.01) RNFL thickness (Table 3). The average and quadrants (superior, inferior, nasal) RNFL thickness were significantly lower in thin cornea group compared to thick cornea group in POAG patients.

There was no statistically significant difference in optic disc area (p = 0.45), horizontal cup/disc ratio (p = 0.15), cup area (p = 0.18), cup volume (p = 0.21), rim area (p = 0.11) and temporal RNFL thickness (p = 0.31) between the two groups (Table 2 and 3).

Table 4 gives the correlation coefficient between OCT parameters (ONH parameters and RNFL thickness) and thin CCT. There was a positive correlation with all OCT parameters. Statistical significance was found in: optic disc area (r = 0.429, p = 0.003), and cup/disc area ratio (r = 0.287, p = 0.05). High statistical significance was found in: horizontal cup/disc ratio (r = 0.472, p < 0.001), VCDR (r = 0.578, p < 0.001), average RNFL (r = 0.796, p < 0.001), superior RNFL (r = 0.665, p < 0.001), inferior RNFL (r = 0.650, p < 0.001), nasal RNFL (r = 0.611, p < 0.001) and temporal RNFL thickness (r = 0.601, p < 0.001).

DISCUSSION

OCT provide objective and reliable data of ONH and RNFL with a high reproducibility in glaucoma and healthy eyes [9].

CCT has been demonstrated as an important risk factor for development and progression of ocular hypertensive to primary open-angle glaucoma patients [10]. The Ocular Hypertension Treatment Study (OHTS) discovered that the risk for development of glaucoma is larger in eyes with thin CCT and lower in eyes with thick CCT [10]. Our study showed a significantly lower mean IOP (p < 0.0001) and CCT (p < 0.0001) in POAG patients with thin cornea compared to patients with thick cornea. Patil et al. [11] demonstrated that the mean CCT in the normal group (554.38 ± 17.67 μm) and the glaucoma group (554.15 ± 16.39 μm) was similar and was significantly lower than the mean CCT in the OHTN group

Table 1. Demographic and clinical characteristics of patients with primary open-angle glaucoma

Parameters	CCT < 540 µm (n = 45) $\bar{x}$ ± SD	CCT ≥ 540 µm (n = 52) $\bar{x}$ ± SD	p
Age (years)	59.93 ± 12.81	54.80 ± 12.91	0.053
Gender (M/F), n	17/28	22/30	0.65
Mean IOP (mmHg)	15.62 ± 2.39	17.92 ± 2.40	< 0.0001
CCT (µm)	512.44 ± 20.39	569.65 ± 22.06	< 0.0001
MD (dB)	-3.72 ± 1.57	-3.22 ± 1.1	0.077

M/F – male/female; IOP – intraocular pressure; CCT – central corneal thickness; p – unpaired t-test; MD – mean deviation

Table 2. Optic nerve head topography parameters classified by central corneal thickness (CCT)

Optic nerve head parameters	CCT < 540 µm (n = 45) $\bar{x}$ ± SD	CCT ≥ 540 µm (n = 52) $\bar{x}$ ± SD	p
Optic disc area (mm^2)	1.72 ± 0.4	1.78 ± 0.36	0.45
Cup/disc area ratio	0.48 ± 0.15	0.42 ± 0.11	< 0.03
Horizontal cup/disc ratio	0.67 ± 0.13	0.63 ± 0.12	0.15
Vertical cup/disc ratio	0.71 ± 0.12	0.69 ± 0.10	< 0.01
Cup area (mm^2)	0.84 ± 0.33	0.76 ± 0.27	0.18
Cup volume (mm^3)	0.21 ± 0.13	0.18 ± 0.10	0.21
Rim area (mm^2)	0.87 ± 0.29	0.97 ± 0.31	0.11
Rim volume (mm^3)	0.12 ± 0.06	0.15 ± 0.05	< 0.01

p – unpaired t-test

Table 3. Retinal nerve fiber layer (RNFL) thickness classified by central corneal thickness (CCT)

RNFL thickness	CCT < 540 µm (n = 45) $\bar{x}$ ± SD	CCT ≥ 540 µm (n = 52) $\bar{x}$ ± SD	p
Average (µm)	102.88 ± 11.04	110.32 ± 10.83	< 0.001
Superior (µm)	118.42 ± 16.76	125.57 ± 15.82	< 0.03
Inferior (µm)	118.44 ± 19.38	126.59 ± 16.93	< 0.03
Temporal(µm)	63.15 ± 9.81	71.47 ± 11.03	0.31
Nasal (µm)	78.33 ± 12.39	84.15 ± 11.16	< 0.01

p – unpaired t-test

Table 4. Optical coherence tomography parameters in relationship to thin central corneal thickness (CCT)

OCT parameters	Correlation coefficient (r)	p
Optic disc area (mm^2)	0.429	0.003
Cup/disc area ratio	0.287	0.05
Horizontal cup/disc ratio	0.472	< 0.001
Vertical cup/disc ratio	0.578	< 0.001
Cup area (mm^2)	0.227	0.126
Cup volume (mm^3)	0.118	0.429
Rim area (mm^2)	0.268	0.069
Rim volume (mm^3)	0.108	0.472
Average RNFL (µm)	0.796	< 0.001
Superior RNFL (µm)	0.665	< 0.001
Inferior RNFL (µm)	0.650	< 0.001
Nasal RNFL (µm)	0.611	< 0.001
Temporal RNFL (µm)	0.601	< 0.001

OCT – optical coherence tomography; RNFL – retinal nerve fiber layer; r – Pearson's correlation coefficient

(568.18 ± 30.52 µm, p < 0.01). Bulut et al. [12] found that the CCT in the POAG group (545.6 ± 29.7 µm) and the healthy control group (551.9 ± 26.2 µm) was significantly higher than the CCT in the normal tension glaucoma group (519.0 ± 25.7 µm, p < 0.001). Marić et al. [13] found in patients with suspected glaucoma significantly lower mean CCT in adults than in children (547 ± 35 µm *vs.* 578 ± 35, p < 0.032).

In the current study ONH parameters showed that the cup/disc area ratio and VCDR were significantly larger and rim volume significantly smaller in POAG patients with thin CCT compared to patients with thick CCT. Anton et al. [14] and Dagdalen and Dirican [9] showed that rim parameters were significantly smaller and C/D ratio significantly greater in glaucomatous eyes than in normal and OHT eyes.

Several studies using OCT showed that the mean RNFL thickness and superior and inferior sector thickness are valuable measurement parameters in the differentiation of glaucoma. Kaushik et al. [15] found that the RNFL in ocular hypertensives with CCT ≤ 555 µm was thinner than in those with thicker corneas. Anton et al. [14] and Dagdalen and Dirican [9] discovered that mean RNFL thickness and superior and inferior RNFL thickness were thinner in eyes with glaucoma, than in eyes with ocular hypertension and normal eyes. Chen et al. [16] found that the most RNFL thickness (except at the nasal quadrant) were significantly lower in preperimetric glaucoma eyes compared to normal eyes. Bulut et al. [12] discovered that the mean RNFL thickness were thinner in normal tension glaucoma group than in POAG and healthy control group. In the present study, the average and quadrants (superior, inferior, nasal) RNFL thickness were significantly lower in thin cornea group compared to thick cornea group in POAG patients.

In our study a significant positive correlation was found between thin CCT and OCT parameters in: optic disc area (p = 0.003), cup/disc area ratio (p = 0.05), horizontal cup/disc ratio (p < 0.001), VCDR (p < 0.001), average RNFL (p < 0.001), superior RNFL (p < 0.001), inferior RNFL (p < 0.001), nasal RNFL (p < 0.001) and temporal RNFL thickness (p < 0.001). In the recent study Öztürker [17] found a significant positive correlation between thin CCT and inferior RNFL thickness (r = 0.353, p < 0.005) in patients with POAG. Wangsupadilok and Orapiriyakul [18] found a significant positive correlation between CCT and RNFL thickness in all quadrants and average RNFL thickness, with highest correlation for average RNFL thickness (r = 0.487, p = 0.001) in POAG patients.

CONCLUSION

POAG patients with thin cornea will probably develop larger glaucoma changes than those with a thicker cornea. Ultrasonic pachymetry measurements of CCT and OCT analysis of ONH topography parameters and RNFL thickness, provide significant information in the early diagnosis and monitoring progression of POAG. It is necessary to perform a larger prospective study in the future to confirm these findings.

Conflict of interest: None declared.

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Повезаност топографских параметара главе оптичког нерва и дебљине слоја нервних влакана ретине са централном дебљином рожњаче код болесника са примарним глаукомом отвореног угла

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САЖЕТАК

Увод/Циљ Истраживали смо повезаност између топографских параметара главе оптичког нерва (ГОН) и дебљине слоја нервних влакана ретине (СНВР) са централном дебљином рожњаче (ЦДР) код болесника са примарним глаукомом отвореног угла.

Методе У ову ретроспективну студију укључено је 97 болесника (97 очију) са примарним глаукомом отвореног угла. Болесници су подељени на групу са тањом рожњачом (ЦДР < 540 *μm*, 45 очију) и дебљом рожњачом (ЦДР ≥ 540 *μm*, 52 ока), које су мерене ултразвучном пахиметријом. Топографска мерења параметара ГОН и дебљине СНВР рађена су оптичком кохерентном томографијом (ОКТ). Резултати су упоређивани са тањом и дебљом ЦДР и корелирани са тањом ЦДР учесника студије.

Резултати Утврђене су статистички значајно ниже вредности интраокуларног притиска ($p < 0,0001$) и ЦДР ($p < 0,0001$) код болесника са танком ЦДР у поређењу са болесницима са дебљом ЦДР. Код параметара ГОН добили смо статистички значајну разлику у групи са танком рожњачом у поређењу са групом са дебљом рожњачом код површине односа *cup/disc* ($p < 0,03$), вертикалног односа *cup/disc* ($p < 0,01$) и волумена *rim* ($p < 0,01$). Пронађена је статистички значајна разлика код дебљине СНВР у групи са танком рожњачом у поређењу са групом са дебљом рожњачом код средње ($p < 0,001$), горње ($p < 0,03$), доње ($p < 0,03$) и унутрашње ($p < 0,01$) дебљине СНВР. Утврђена је статистички значајна позитивна корелација танке ЦДР и параметара оптичке кохерентне томографије код површине *optic disc* ($r = 0,429$, $p = 0,003$), површине односа *cup/disc* ($r = 0,287$, $p = 0,05$), хоризонталног односа *cup/disc* ($r = 0,472$, $p < 0,001$), вертикалног односа *cup/disc* ($r = 0,578$, $p < 0,001$), средње дебљине СНВР ($r = 0,796$, $p < 0,001$), горњег СНВР ($r = 0,665$, $p < 0,001$), доњег СНВР ($r = 0,650$, $p < 0,001$), унутрашњег СНВР ($r = 0,611$, $p < 0,001$) и спољашњег СНВР квадранта ($r = 0,601$, $p < 0,001$).

Закључак Болесници са примарним глаукомом отвореног угла и тањом рожњачом вероватно ће развити веће глаукомне промене од оних са дебљом рожњачом. Ултразвучном пахиметријом мерена ЦДР и оптичком кохерентном томографијом анализирани топографски параметри ГОН и дебљина СНВР пружају значајне информације у раној дијагнози и праћењу прогресије код примарног глаукома отвореног угла.

Кључне речи: интраокуларни притисак; ултразвучна пахиметрија; оптичка кохерентна томографија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Abnormalities in the thickness of the retinal ganglion cell / inner plexiform layer in age-related macular degeneration

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SUMMARY

Introduction/Objective The study aims to analyze the thickness of both the ganglion cell layer and the inner plexiform layer (GCL + IPL) among patients suffering from dry and wet form of age-related macular degeneration (AMD).

Methods One hundred ninety-five patients with AMD participated in the study, along with 94 healthy individuals (mean age 75.2 ± 7.8 years; range 55–86). They were divided into three groups: the first group, or group I, included 100 patients suffering from wet AMD; the second group, or group II, included 95 patients afflicted with dry AMD; the final 94 patients made up the control group, group III, of healthy individuals without systemic or ocular diseases. Measurements such as the average macular thickness, the average and minimum GCL + IPL thickness, and the GCL + IPL thickness in all six sectors were obtained by Cirrus spectral-domain optical coherence tomography (SD-OCT, Carl Zeiss Meditec, Inc., Dublin, CA, USA). SPSS version 20.0 was used to analyze the data, while the level of statistical significance was set at $p < 0.05$.

Results In the case of patients with wet AMD, the average value for GCL + IPL thickness was 43.13 µm, for patients with dry AMD the value was 66.73 µm, and the average thickness measured for the control group was 86.23 µm. There was a statistically significant difference between the average GCL + IPL and minimum GCL + IPL thicknesses between the groups ($p < 0.001$). Lower values were noted for patients with wet AMD ($p < 0.001$) than those with dry AMD. In the latter, the average GCL + IPL and the minimum GCL + IPL thicknesses were lower than those of the healthy participants, at a level of statistical significance ($p < 0.001$).

Conclusion Participants with AMD exhibited thinner GCL + IPL than the healthy participants, as did the participants with wet AMD when compared to the participants with dry AMD.

Keywords: dry AMD; wet AMD; ganglion cell layer; ganglion cell complex

INTRODUCTION

The onset of blindness in developed countries is frequently caused by age-related macular degeneration (AMD) [1, 2, 3]. The basic changes which lead to the disease mostly affect the outer retinal layers, the Bruch's membrane, and the choriocapillaris. AMD leads to degeneration of the photoreceptors and retinal pigment epithelium of the macula [4–8]. The later stages of the disease can develop into geographic atrophy (GA) or neovascular AMD [9–14].

However, lately, any changes affecting the inner layers of the retina among patients suffering from AMD have been the focus of further study. Consequently, a great deal of importance has been paid to inner retinal layer analysis, especially to the ganglion cell complex (GCC), that is, the ganglion cell layer – or the inner plexiform layer (GCL + IPL) in particular. The layer can be obtained through segmentation, by using optical coherence tomography (OCT) [15].

The three layers contained within the inner retina make up the retinal ganglion cells complex. They include the following: the retinal nerve fiber layer (RNFL), encompassing the ganglion cell axons; the GCL, consisting of the ganglion cell bodies; and the IPL, made up of the ganglion cell dendrites [15]. The GCC is not necessarily solely limited to making differential diagnoses or managing glaucoma. It is important to note that its application extends to multiple neurological and retinal conditions [16, 17].

In clinical practice, an AMD diagnosis is usually made following an ophthalmoscopic examination of the macula, color fundus photography, an Amsler grid, visual field testing with automatic microperimetry in scotopic conditions, a test for the macular threshold, contrast-sensitivity, and fluorescein angiography [18, 19].

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Another essential diagnostic tool is OCT. Its advantage is that is provides not only real-time, but also objective and reproducible retinal thickness measurements, as well as morphological measurements [20, 21]. In the detection of any structural damage to the retina, OCT is known to researchers as the gold standard. It also measures macular GCC thickness, which represents the combined thickness of RNFL, the GCL + IPL around the macula. The Cirrus spectral-domain optical coherence tomography (SD-OCT, Carl Zeiss Meditec, Inc., Dublin, CA, USA) incorporates the latest ganglion cell analysis algorithm, which precisely delineates only the macular GCL + IPL, excluding the RNFL. Therefore, any effect on the total GCC which could result from possible changes to the RNFL is precluded [22, 23, 24].

The purpose of this study was to analyze macular GCL + IPL thickness in patients with the dry and wet form of AMD. These values were compared to those obtained from measurements of a control group of patients, so as to assess any potential impact of AMD on the inner layers of the retina.

METHODS

A prospective, nonrandomized, observational monocentric study was conducted on 195 patients with AMD and 94 healthy persons between May 2016 and May 2017 at the Maja Clinic Special Hospital for Ophthalmology, Niš, Serbia. The study was approved by the Clinic Ethics Committee. The patients included in the study were divided into three groups: the first group, or group I, included 100 patients suffering from wet AMD; the second group, or group II, included 95 patients afflicted with dry AMD; the final 94 patients made up the control group, or group III, consisting of healthy patients without systemic or ocular diseases. After presenting at the Clinic, the participants with noted AMD were enrolled consecutively; the control group was recruited from a population of normal, healthy, sex- and age-matched individuals. The implemented procedure followed the tenets of the Declaration of Helsinki. Each patient provided informed consent following an explanation of the nature of the study, and any possible consequences thereof.

The inclusion criteria were as follows: age over 55 years and retinal changes classified as stages of the AMD. Patients with end-stage disease, GA, and end-stage AMD were excluded from the study.

The exclusion criteria included the following: any ocular disease with a possible confounding effect on the assessment of the retina, except AMD (retinal vessel occlusion, glaucoma, diabetic retinopathy, retinal dystrophies, and uveitis), as well as any previous or any concomitant therapy the patient might have been undergoing to treat their AMD (intravitreal steroids, anti-vascular endothelial growth factor, ocular surgery, laser coagulation etc.). In addition, patients with any neurologic disease were excluded from the study.

The study procedures included a baseline visit, followed by regular study visits, and a full ophthalmic and fundus examination, including multimodal retinal imaging as required by standard operating procedures. These procedures required the use of a Cirrus SD-OCT device (model 4000, software version 6.0). The thickness of the macular GCL + IPL was measured using the ganglion cell analysis algorithm included in the aforementioned device. The following measurements were taken: the average and minimum thickness of the GCL + IPL, along with the thickness of the six sectors (superotemporal, superior, superonasal, inferonasal, inferior, inferotemporal) starting from the elliptical annulus which is centered on the fovea. Macular image acquisition was obtained once mydriasis was achieved following the administration of 1% tropicamide eye drops. The data collected from the healthy patients were also analyzed. They were subject to the following criteria: (1) no ocular pathology, (2) no history of ocular surgery including intravitreal injections, (3) normal computerized visual field – standard automated perimetry finding [24–2 Swedish Interactive Threshold Algorithm (SITA), Humphrey Field Analyzer II, Carl Zeiss Meditec], (4) intraocular pressure ≤ 21 mmHg measured by Goldmann applanation tonometer, (5) no systemic or/and neurological disease, and (6) best-corrected visual acuity 20/20, and refractive error within ± 0.5 D.

Following the baseline visit, the control group of healthy participants needed to return for a follow-up visit after one year. Since the study is longitudinal, in order for the data from the control group to be included in the analysis, the participants needed to complete at least two visits over the span of a single year.

Statistical analysis

We classified the eyes into three groups based on their clinical pattern: neovascular (wet) AMD, dry AMD, and control subjects. Kruskal–Wallis and Man–Whitney nonparametric statistical tests for independent data were used to compare the value of GCL + IPL in neovascular (wet) and dry AMD and in the control group. The quantitative values calculated in the study were expressed as means and standard deviations. The level of statistical significance for the statistical calculations was set at $p < 0.05$. The confidence interval was set at 95%. IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for all the data analyses.

RESULTS

One hundred eyes of 100 patients (mean age at presentation 75.2 ± 7.8 years, range 55–86 years; male *vs*. female 51/49) with signs of wet AMD from the base of our OCT study were compared with 95 patients with dry AMD (mean age 70.1 ± 7.1 years, range 55–83 years; male *vs*. female 48/47). The control group comprised 94 age- and sex-matched patients (mean age 73.3 ± 7.3, range 55–85 years; male *vs*. female 47/47). The necessary demarcation of the superior sectors was done from the nasal to the temporal sector. Consequently, the superior nasal sector of GCL + IPL was marked GC1, the superior one GC2,

and the superior-temporal one GC3. The demarcation of the inferior sectors was also done from the temporal to the nasal sector, which meant that the inferior temporal sector was marked GC4, the inferior one GC5, and the inferior-nasal one GC6.

The Kruskal–Wallis nonparametric statistical test showed a statistically significant difference for the individual thickness of each segment of the GC complex (GC1–GC6) between the three groups ($p < 0.001$) –wet AMD, dry AMD, and the control group of healthy patients. By using the Mann–Whitney tests we demonstrated that separate thicknesses of sectors GC1–GC6 were higher in healthy patients ($p < 0.001$) than in both AMD groups, as well as in the group of dry AMD patients than in the wet AMD ($p < 0.001$) group – the detailed values are reported in Table 1. As indicated, the control group had a thicker average GCC (86.23 μm), the dry AMD group had a thickness of 66.73 μm, and the wet AMD group had a thickness of 43.13 μm.

Table 1. Mean ganglion cell layer and inner plexiform layer (μm) in wet, dry age-related macular degeneration and control subjects

Sector of GCL + IPL	AMD wet n = 100	AMD dry n = 95	Control subjects n = 94	p
GC1	44.47	67.55	86.34	< 0.001
GC2	42.76	64.7	86.55	< 0.001
GC3	43.46	66.1	86.45	< 0.001
GC4	43.26	66.38	86.12	< 0.001
GC5	44.38	66.13	86.62	< 0.001
GC6	42.04	67.55	86.31	< 0.001
GCL avg.	43.13	66.73	86.23	< 0.001
GCL min.	40.03	60.57	82.49	< 0.001
CFT	292.07	195.94	255.43	< 0.001

GCL + IPL – ganglion cell layer and inner plexiform layer; GC1 – superior nasal sector of GCL + IPL; GC2 – superior sector of GCL + IPL; GC3 – superior-temporal sector of GCL + IPL; GC4 – inferior temporal sector of GCL + IPL; GC5 – inferior sector of GCL + IPL; GC6 – inferior-nasal sector of GCL + IPL; GCL avg. – ganglion cell layer and inner plexiform layer average; GCL min. – ganglion cell layer and inner plexiform layer minimum; CFT – central foveal thickness

A statistically significant difference was noted between the values for average GCL + IPL between the groups ($p < 0.001$). It was thinner in patients with wet AMD ($p < 0.001$) than inthose with dry AMD. A statistically significant difference was noted between the values of average GCL + IPL for the dry AMD group and the group of healthy patients ($p < 0.001$).

Statistically significant differences between the groups were also determined for minimum GCL + IPL thickness ($p < 0.001$). The results indicated it was thinner among the patients of the wet AMD group ($p < 0.001$) than among the patients of the dry AMD group. A statistically significant difference for minimum GCL + IPL was noted between the patients of the dry AMD group and the healthy group of patients ($p < 0.001$), with lower thickness recorded for the former.

A statistically significant difference for central foveal thickness was noted for all three groups. The lowest thickness was noted for the dry AMD group, 195.94 μm, followed by the control group – 255.43 μm, and the greatest thickness was noted in patients with wet AMD – 292.07 μm.

DISCUSSION

The introduction of OCT started a revolution in the research on inner retinal layers in patients with AMD. Special attention had to be focused on three layers: the IPL, the GCL, and the RNFL, which form the GCC [2, 21]. Software upgrades for the SD-OCT systems have been developed and made available to the public to keep up with the progress of research into the thickness of the GCL [22].

The segmentation of the GCC, which allows researchers to measure the thickness of this section alone, as well as track any thickness changes that take place over time, enabled them to recognize the importance of GCC when establishing diagnoses and monitoring any changes that occur in patients afflicted with various diseases of the retina, the macula, and the optic nerve. The importance of GCC segmentation and the need to perform it have been proven in multiple studies [23, 24]. It is now possible to segment the macular GCL + IPL, with the exclusion of the remaining RNFL, by using the ganglion cell analysis algorithm which is now a constituent part of the Cirrus HD-OCT (Cirrus Version 6.0) [25]. Consequently, any changes to the RNFL will not affect the entire GCC.

So far, only a few studies have investigated GCL + IPL thickness in AMD. Zucchiatti et al. [26] investigated exactly the same GCL + IPL as we did. Their results showed that mean GCC thickness was higher in the control group (79.9 ± 5.5 μm), becoming progressively thinner in advanced AMD forms (neovascular AMD had a mean GCC thickness of 53.8 ± 16.9 μm while atrophic AMD had a mean of 50.4 ± 17.9 μm). Compared to the results of our study, the values of GCC thickness are almost the same.

It is also of great importance to monitor the changes in GCL + IPL thickness after multiple anti-vascular endothelial growth factor (anti-VEGF) therapy treatments to observe if there is an additional reduction in thickness of this layer, or whether anti-VEGF therapy does not adversely affect GCL + IPL thickness. There are a few studies investigating the effect of intravitreal anti-VEGF injections on RNFL and GCC, but the results are controversial [27, 28]. It is important to point out that the GCL + IPL layer is thinned out by the disease itself before any application of the VEGF treatment and it should be analyzed among patients whose visual acuity did not improve despite aggressive anti-VEGF treatment. Further studies should focus on the investigation of GCL + IPL thickness after multiple applications of anti-VEGF treatment.

Patients with dry AMD are expected to have thinner GCL + IPL compared to patients with wet AMD, which was not confirmed in this study. More studies are required for a better understanding of retinal structural changes in neovascular AMD that occur as a consequence of the natural course of the disease.

Lee et al. [29], analyzing the GCL + IPL and RNFL among patients with dry AMD, reached the conclusion that in in this particular case, the thickness of the GCL + IPL and the RNFL were not as high as those measured in control eyes. The results of this study also indicate that there is a negative correlation between the thickness of

the average GCL + IPL and the drusen area. In addition, patients suffering from GA, caused by AMD, experience significant GCL loss [30]. In the aforementioned study, the authors were able to conclude that ganglion cell death could be followed by axonal loss, and that increased macular RNFL volumes are not indicative of GCL volume [30]. Borelli et al. [31] analyzed the GCC of 68 eyes with intermediate AMD which exhibited GCC thinning (the average and minimum GCC thicknesses were thinner in AMD patients than in healthy controls (69.54 ± 9.30 μm and 78.57 ± 6.28 μm, respectively), which supports the concept of postreceptor retinal neuronal loss as a contributing factor to retinal thinning in intermediate AMD.

It is important to point out that the presence of thinning not only in GCL + IPL but also in RNFL imposes the following question: how are the structural changes in patients with both glaucoma and AMD supposed to be followed, since both diseases result in the thinning of both GCL + IPL and RNFL. Rimayanti et al. [32] concluded that there is damage to the inner retinal layers in eyes with AMD. Even if this is not the topic of interest in our current study, it is useful to have in mind that RNFL thickness significantly correlates with glaucoma in AMD eyes. RNFL thickness can be a useful parameter for differentiating eyes with AMD from eyes with both AMD and glaucoma [32].

CONCLUSION

Changes to the inner macular layer are a consequence of both dry and wet AMD. These changes take the form of a reduction in the thickness of the macular GCL + IPL. Patients with cases of wet AMD have a thinner GCL + IPL compared to patients with cases of dry AMD. This excludes any patients suffering from GA.

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Промене дебљине слоја ганглијских ћелија и унутрашњег плексиформног слоја код болесника са сенилном дегенерацијом жуте мрље

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САЖЕТАК

Увод/Циљ Циљ студије је био да се анализирају промене дебљине слоја ганглијских ћелија и унутрашњег плексиформног слоја СГЋ + УПС код болесника са сувим и влажним обликом сенилне дегенерације макуле (СДМ) у односу на контролну групу.

Метод Студија је спроведена на 195 болесника са СДМ и 94 здраве особе (средња старосна доб 75,2 ± 7,8 година; опсег 55–86 година). Болесници су подељени у три групе: група I – 100 болесника са влажном СДМ, II група – 95 болесника са сувом СДМ и контролна група III – 94 испитаника без системских или очних болести. Испитивање је вршено помоћу *Cirrus SD-OCT* (*Carl Zeiss Meditec, Inc*., Даблин, Калифорнија, САД). Мерене су просечна дебљина макуле, као и просечна и минимална дебљина СГЋ + УПС и дебљина СГЋ + УПС у свих шест сектора. Статистичка анализа извршена је коришћењем *SPSS* верзије 20.0 применом одговарајућих статистичких метода. Вредност $p < 0,05$ се сматрала статистички значајним резултатом.

Резултати Просечна дебљина СГЋ + УПС код болесника са влажном формом СДМ износила је 43,13 *μm*, код болесника са сувом СДМ била је 66,73 *μm*, а у контролној групи 86,23 *μm*. Просечне вредности СГЋ + УПС и вредности минималне дебљине СГЋ + УПС статистички се значајно разликују између група ($p < 0,001$) и биле су тање у влажној форми СДМ ($p < 0,001$) него код суве форме СДМ. У групи суве форме СДМ, просечна дебљина СГЋ + УПС и минимална дебљина СГЋ + УПС су статистички тање него код здравих особа ($p < 0,001$).

Закључак СДМ одликује истањење СГЋ + УПС. Болесници са влажном формом СДМ имају тањи СГЋ + УПС у поређењу са болесницима са сувом формом СДМ.

Кључне речи: сува СДМ; влажна СДМ; слој ганглијских ћелија; комплекс ганглијских ћелија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

p16 status of oropharyngeal and oral cavity squamous cell carcinomas – a single institution experience

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SUMMARY

Introduction/Objective New World Health Organization Classification of Head and Neck Tumors from 2017 has introduced significant changes, mainly considering tumors in the oropharyngeal region. New entities of HPV-positive and -negative squamous cell carcinomas have been acknowledged, not only based on the presence of an active viral infection and different tumor markers expression, but also because of their different histopathology, staging assessment, and prognosis. A retrospective study has been conducted, in order to determine p16 positivity in squamous cell carcinomas in oropharynx and in the oral cavity, and to see whether they differ in sex and age distribution.

Methods The presence of viral infection was verified based on p16 immunochemistry staining, p16 being the surrogate marker for HPV infection. A total of 177 cases of squamous cell carcinomas in the oropharynx and the oral cavity, found in the archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Centre of Serbia, have been revised.

Results Out of 177 cases, 50 (28.2%) were p16-positive. Compared with carcinomas in the oral cavity, p16 carcinomas were significantly more common in the oropharynx (34.3% in the oropharynx, compared to 10.3% in the oral cavity). Carcinomas in both regions were mostly associated with male sex (88.1% of all cases were in males), but p16 positivity was more common in females (11 out of 21 cases, 52.4%). The most common location of p16-positive carcinomas were palatine tonsils (41.03% of tonsillar carcinomas were p16-positive).

Conclusion P16-positive squamous cell carcinomas were the most numerous in the oropharynx, i.e. palatine tonsils, and were more common in females.

Keywords: oropharynx; oral cavity; human papilloma virus; squamous cell carcinoma

INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC) is a medical problem on the rise. In the new World Health Organization (WHO) classification from 2017, oropharyngeal tumors are found to be so important that they are separated in a new chapter, being divided from tumors in the oral cavity, in which they were incorporated in previous classification from 2005 [1, 2]. These tumors are now classified as p16-positive, associated with *human papillomavirus* (HPV) infection, and p16-negative, associated with long-term cigarette smoking and alcohol abuse [3–7].

Oral HPV infection has been proven as a significant factor in the genesis of squamous cell carcinoma (SCC) in this region [4]. Infection is usually transmitted by urogenital sexual contact, so it is mostly found in young, sexually active adults [4, 5]. In the past few years, there has been a dramatic increase in the incidence of OPSCC attributed to HPV infection, mainly in developed countries [8, 9, 10]. Most common viral subtype found in these tumors is HPV 16, which has a high tumorous potential [5, 11].

P16-positive SCC originates from malignant transformation of the oropharyngeal epithelium, caused by viral replication within the cells. Most convenient for HPV replication is tonsillar crypt epithelium, so generally these carcinomas occur most commonly in the palatine tonsils. Cancer grows by imitating tonsillar crypt architecture, so it differs from p16-negative carcinomas not only by etiology, but also by histopathology [1, 2, 5]. Given that the cancer arises not from the superficial squamous epithelium but from the reticulated epithelium which coats the tonsillar crypts, most of the cancers are nonkeratinizing and of basaloid appearance [2, 5].

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Consequently, grading of tumors into well differentiated, poorly differentiated, and undifferentiated is no longer applicable, so the new WHO Classification discourages the practice of grading HPV-positive OPSCC. Ultimately, p16-positive OPSCC have been related to a significantly better prognosis in comparison to p16-negative OPSCC, as well as to a lower risk of cancer recurrence [12, 13, 14].

There is no specific test for proving the active HPV infection in the oropharynx, so expression of the p16 protein in cells is used as a reliable surrogate marker [5, 15]. p16 is a tumor suppressor protein that inhibits cyclin-dependent kinase 4A, which further phosphorylates the retinoblastoma (Rb) protein and allows the cell to progress from G1 to S phase of the cell cycle. By a feedback mechanism, Rb normally inhibits the expression of p16. Carcinogenesis of most of the head and neck carcinomas involves inactivation of the tumor suppressor proteins, including p16. However, viral E7 protein produced in HPV infection leads to functional inactivation of the Rb protein, and consequential overexpression of the p16 protein, which can be used to detect these tumors by immunohistochemical staining methods [5, 15].

The aim of the study is to determine p16 positivity of SCC in the oropharynx and the oral cavity, as well as to determine differences in age and sex distribution.

METHODS

A retrospective study has been conducted based on cases found in archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery (Clinical Centre of Serbia), from January 1, 2013 to October 1, 2017. A total of 173 cases of SCC in the oropharynx and the oral cavity were found, as well as four cases of primarily found metastasis of this carcinoma in the neck lymph nodes.

All the samples were standardly processed in the Clinic's laboratory (formalin-fixed, paraffin-embedded and cut into 4 μm sections), and sent for staining to the Immunohistochemistry Laboratory of the Institute of Pathology, Faculty of Medicine, Belgrade. A monoclonal p16 antibody was used (anti-mouse, ready-to-use; Ventana Medical Systems, Inc., Oro Valley, AZ, USA). Samples were stained partly manually, partly in an immunostainer (BenchMark Special Stains, Ventana Medical Systems, Inc.) following the standard laboratory protocols. Detection involved a two-stage UltraVision LP detection system (Thermo Fisher Scientific, Waltham, MA, USA), which included secondary (Primary Antibody Enhancer) and tertiary (large volume HRP polymer) antibodies. The reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride chromogen. The samples were then stained with hematoxylin according to standard protocols and evaluated under a microscope. Samples with both nuclear and cytoplasmic immunoreactivity of more than 75 % of tumor cells were considered p16-positive (Figure 1). All histopathological procedures including tissue manipulations were a part of a routine diagnostic procedure that is applied for our patients.

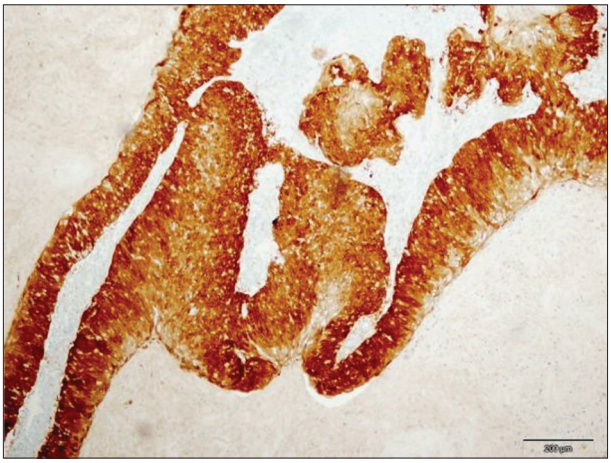

Figure 1. An example of p16-positive staining sample: intensive nuclear and cytoplasmic positivity in more than 75% of tumor cells (streptavidin-biotin, × 400)

The data was processed in IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) using the methods of descriptive and analytic statistics. Based on the type of data, the following tests have been used: Fisher's exact test, χ^2 test, ANOVA, student's t-test. The results were considered significant for p-values less than 0.05.

The paper was approved by the institutional review board.

RESULTS

Out of 177 patients with SCC, 156 (88.1%) were male and 21 (11.9%) were female. The average age was 62.82 ± 10.19 years; the youngest patient was 28, and the oldest one 93 years old. The most common localization of all SCC was the palatine tonsil, in 78 (44.1%) patients. Other most common localizations were as follows (Figure 2): the tongue base (14.1%), the mobile tongue (11.3%), and the posterior pharyngeal wall (11.3%).

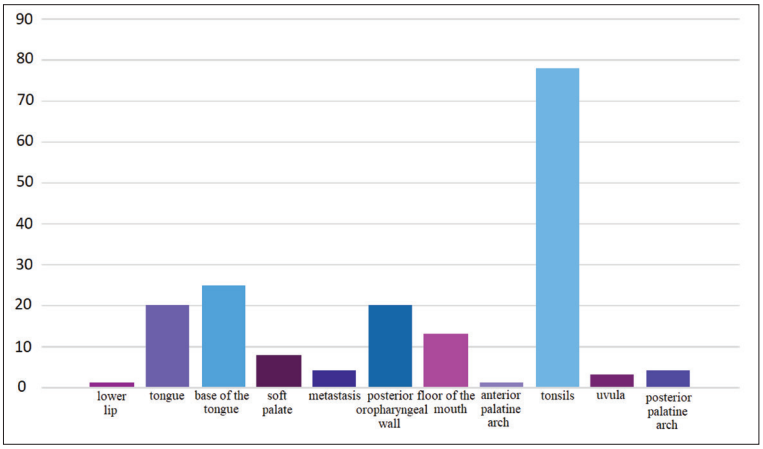

Figure 2. Site distribution of squamous cell carcinomas in the oropharynx and the oral cavity

All the cases were divided in three groups. The first group (Table 1) were OPSCC, which included tumors in the tonsils, the soft palate, the tongue base, the posterior oropharyngeal wall, and the uvula (n = 134). The second group (Table 2) consisted of the oral cavity SCC, including tumors on the lips, floor of the mouth, the mobile tongue, the anterior and posterior palatine arches (n = 39). The third group was made out of four cases of primarily found metastasis of SCC in the lymph nodes of the neck region (n = 4).

The groups were compared regarding the p16 positivity, age and sex distribution (Tables 1 and 2). Sex distribution among the groups was not significantly different (p = 0.179), and neither was age distribution (p = 0.541). p16 positivity was most commonly found in the oropharynx (34.3% of the cases); in the oral cavity, 10.3% of SCC were p16-positive, whereas all four of the metastases were p16-negative. Statistically significant difference was found in the p16 positivity distribution among the groups (p = 0.006, Table 3). Out of all p16-positive SCC, 92% were in the oropharynx and only 8% in the oral cavity.

In the oropharynx, there were 46 p16-positive cases, out of which 37 (80.4%) were male and nine were female (19.6%). The average age of p16-positive patients was 64.83 ± 10.68 years. The other 88 cases were p16-negative, with 84 males (95.5%) and four females (4.5%), and the average age was 62.41 ± 7.8 years.

In total, there were 50 (28.2%) p16-positive cases in both regions. Out of all males, 39 (25%) had p16-positive SCC, whereas 11 (52.4%) females had p16-positive SCC. P16 positivity was significantly more common in females (p = 0.009, Table 4). The mean age of p16-positive patients was 65.12 ± 10.71 years, and the mean age of p16-negative patients was 61.92 ± 9.87 years. p16-positive and -negative patients did not significantly differ in the age distribution (p = 0.06).

Table 1. Distribution of squamous cell carcinoma in various parts of the oropharynx

Localization	Frequency
Tonsils	78 (58.2%)
Root of the tongue	25 (18.7%)
Soft palate	8 (6%)
Posterior oropharyngeal wall	20 (14.9%)
Uvula	3 (2.2%)

Table 2. Distribution of squamous cell carcinoma in the oral cavity

Localization	Frequency
Lower lip	1 (2.6%)
Tongue	20 (52.3%)
Floor of oral cavity	13 (33.3%)
Palatoglossal arch	1 (2.6%)
Palatopharyngeal arch	4 (10.3%)

Table 3. Distribution of p16-positive and negative squamous cell carcinoma in the oropharynx and the oral cavity (p = 0.006, χ^2 test)

p16 status	Oropharynx (n = 134)	Oral cavity (n = 39)
p16+	46 (34.3%)	4 (10.3%)
p16-	88 (65.7%)	35 (89.7%)

Table 4. Distribution of p16 positivity as to sex (p = 0.009, χ^2 test)

p16 status	Male (n = 156)	Female (n = 21)
p16+	39 (25%)	11 (54.4%)
p16-	117 (75%)	10 (47.6%)

DISCUSSION

P16-positive OPSCC have been acknowledged as new entities based on their significant differences in regard to p16-negative SCC in this region. Correlation with HPV infection causes their predominant occurrence in the palatine tonsils and their characteristic histological appearance, lower mean age of patients affected, and better prognosis and lower recurrence rates in comparison to SCC related to smoking and alcohol abuse [1, 2, 5–9, 12–16].

In our study, an expected higher incidence of p16-positive SCC in the oropharynx, followed by the oral cavity, has been shown (34.3% in the oropharynx compared to 10.3% in the oral cavity). However, in our population, p16-negative SCC prevailed, in contrast to the USA, Sweden, and the Netherlands [8, 9, 10]. Given that these are more developed countries, leading the anti-smoking campaign for many years, it is understandable why there is a significant fall in the incidence of p16-negative SCC in the last few decades. On the other hand, more liberal sexual behavior could explain higher incidence of HPV infection in these countries.

Considering the lack of data on incidence of p16-positive SCC in our country prior to 2013, we could not determine whether there has been a serious increase in its incidence. A study conducted in the USA showed an increase in the incidence of p16-positive SCC of 225%, in the 1988–2004 period, whereas a study from Sweden determined a rise of 295% from the 2000–2002 period to the 1970–1979 period [8, 9]. Meta-analysis done based on data from Europe and North America showed a significant rise in the number of p16-positive SCC, from 47.7% before 2000 to 72.2% in the 2005–2009 period [16].

The mean age of patients with p16-positive OPSCC in our study did not significantly differ from the mean age of patients with p16-negative carcinomas, though p16-positive patients were on average older than the p16-negative ones (64.83 ± 10.67 years for positive, and 62.41 ± 7.87 years for negative), which is different from statistics in other countries. Studies conducted in the USA and Sweden concluded that patients with p16-positive SCC were significantly younger than those with p16-negative SCC, which is expected given the risk factors [8, 9].

Both p16-positive and -negative SCC were found significantly more often in males (90.3% of all cases were male, and only 9.3% were female), which is in accordance with other studies [8, 9, 10].

p16 positivity in our study was more often found in females; 69.23% of the females had p16-positive OPSCC, in comparison to 30.58% of the males. This information correlates with study conducted in Stockholm, where 96% of the females had a p16-positive carcinoma, in comparison to 81.58% of the males [9].

In sites other than oropharynx and oral cavity, routine p16 immunostaining is not recommended [17]. In selected patients with enlarged level II/III lymph nodes,

p16 immunohistochemistry may be considered since HPV-associated OPSCC often present with large cervical metastases with occult primary carcinoma.

CONCLUSION

p16-positive SCCs are most common in the oropharynx, namely in the palatine tonsils, and are more commonly found in females.

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Статус *p*16 сквамоцелуларних карцинома орофаринкса и усне дупље – искуство наше институције

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САЖЕТАК

Увод/Циљ Нова класификација тумора главе и врата Светске здравствене организације из 2017. године унела је значајне промене, углавном везане за регију орофаринкса. Уведени су нови ентитети *HPV* позитивних и негативних сквамоцелуларних карцинома, не само на основу присуства активне вирусне инфекције и експресије различитих туморских маркера већ и због своје различите хистопатологије, процене стадијума болести и прогнозе. Спроведена је ретроспективна студија са циљем да се одреди *p*16 позитивност у сквамоцелуларним карциномима орофаринкса и усне шупљине, као и како би се утврдило да ли постоји разлика међу различитим половима и старосним групама.

Методе Присуство вирусне инфекције је потврђивано на основу имунохистохемијске анализе на *p*16, који је маркер за инфекцију хуманим вирусом папилома. Увидом у архиву Патохистолошке лабораторије Клинике за оториноларингологију и максилофацијалну хирургију Клиничког центра Србије прегледано је 177 случајева сквамоцелуларних карцинома орофаринкса и усне шупљине.

Резултати Од 177 случајева, 50 (28,2%) случајева је било *p*16 позитивно. У поређењу са карциномима усне шупљине, карциноми *p*16 су били значајно чешћи у орофаринксу (34,3% у орофаринксу у поређењу са 10,3% у усној шупљини). Карциноми у обе регије су били чешћи код припадника мушког пола (88,1% свих случајева били су мушког пола), али је *p*16 позитивност била знатно чешћа код женских болесника (11 од 21 случаја, 52,4%). Најчешћа локализација *p*16 позитивних карцинома били су непчани крајници (41,03% карцинома крајника било је *p*16 позитивно).

Закључак *p*16 позитивни сквамоцелуларни карциноми били су најбројнији у орофаринксу (тачније у непчаним крајницима) и чешћи су били код женског пола.

Кључне речи: орофаринкс; усна шупљина; хумани вирус папилома; сквамоцелуларни карцином

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative analysis of measuring the body fat percentage by anthropometric methods and bioimpedance

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SUMMARY

Introduction/Objective Body fat percentage (BFP) is the most reliable indicator of the nutritional status. For clinical practice, it is important but also insufficiently examined whether the determination of BFP should be relied exclusively on the latest methods or whether classical anthropometric methods are also reliable. The aim was to investigate the correlation between the results of BFP measuring using a contemporary method of bioimpedance and classic methods of skin fold thickness (SFT) and body mass index (BMI).

Method There were 279 patients of the Dietetic Counseling Center of the Institute for Public Health in Niš who were included in the research during 2015. BFP was determined using three classic anthropometric methods: SFT over the triceps, SFT over the scapula, and BMI. OMRON BF 302 apparatus was used for BFP measuring using the bioimpedance method.

Results Using single-factor analysis of variance we found a statistically significant difference between the mean values of the BFP obtained with bioimpedance and those obtained with anthropometric methods ($F = 24.19$, $p < 0.05$). *Post hoc* analysis revealed a statistically significant difference between the BFP determined with bioimpedance and SFT over the triceps and the scapula, while the anthropometric method based on BMI gave the results similar to those from bioimpedance.

Conclusion We show that the most reliable anthropometric method of determination of BFP is that based on BMI, as its results correlate best with those obtained with a contemporary method of bioimpedance.

Keywords: body fat percentage; BMI; bioimpedance

INTRODUCTION

Body fat percentage (BFP) as a part of the overall body weight gives the most reliable assessment of nutritional status [1]. There are several contemporary methods of determining BFP: bioimpedance, hydro densitometry, air-displacement plethysmography, dual-energy X-ray densitometry, computerized tomography, nuclear magnetic resonance, and near-infrared [2–10].

For this research, we used a bioimpedance method as non-invasive, relatively simple electrical conductivity method based on tissue properties to provide resistance to low-intensity electric current flow. Under the influence of impulses of a low-dose safe alternating current (800 μA), the cells and tissues provide resistance or an electrical bioimpedance that depends on the tissue structure and the frequency of the signal used. Therefore, the frequency response of the electrical impedance of biological tissues is greatly under the influence of their physiological and physicochemical status and varies from subject to subject. It varies from tissue to tissue in a particular subject, as well as with a change in the health status depending on the physiological and physicochemical changes, which occur in the tissue. Non-fatty tissue rich in electrolytes and water (73%) is a good electrical conductor, whereas fatty tissue poor in electrolytes and water (14%) shows great resistance and is a weak conductor [2, 3, 4]. The bioimpedance analysis could also be useful in the planning of physical activity for overweight/obese children and adolescents [11]. The coronavirus disease of 2019 (COVID-19) pandemic has showed that the timely identification and correction of undernutrition also have the potential to improve outcomes of the disease cost-effectively. Practical steps to improve nutritional status at a time when hospital services are particularly stretched are also important [12]. The clinical relevance of the anthropometric data on patients obtained by the bioimpedance is also confirmed [13].

Contemporary methods of BFP measurements are accurate but also expensive, and the research question is whether classic methods based on skin fold thickness (SFT) and on BMI should be abandoned in a clinical practice.

The aim of this investigation was to examine the correlation between the results of BFP measurements obtained with classic anthropometric methods of SFT and BMI and one contemporary method – bioimpedance. The working hypothesis of the research was that some of the classic methods of BFP measurement correlate strongly and positively with the

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contemporary method of bioimpedance and that it can be further recommended for clinical practice.

METHODS

Sample

There were 279 patients of the Dietetic Counseling Center of the Institute for Public Health in Niš who were included in the research during 2015. The inclusion criteria for the study were the following: age between 18 and 59 years, BMI greater than 25, and the absence of chronic illnesses. This information was obtained from the patients' medical records.

Body fat percentage measurements

Body height and body weight and SFT over the triceps and the scapula were measured. The SFT was determined using a mechanical caliper (John Bull British Indicators Ltd). Also, BFP was determined in all the examinees using the OMRON BF 302 apparatus (OMRON Healthcare Co., Ltd., Kyoto, Japan) based on bioimpedance. Trained personnel performed all measurements three times and the mean values were calculated. The examinees were advised not to drink diuretics seven days before the measurement, not to drink alcoholic drinks two days prior to measurements, not to exercise intensively 24 hours prior to measurements, and not to drink any fluids four hours before the measurements.

The BFP determination using classic anthropometric measurements was calculated in three ways: 1) based on SFT over the triceps; 2) based on SFT over the scapula, and 3) based on the BMI. For these three methods, we used the following formulas:

1) $D1 = 1.0923 - 0.0202 \times SFT_t$; $F1 = (4.201 / D1 - 3.813) \times 100$ [1]
   SFT_t – skin fold thickness over triceps;
   D1 – specific body density based on Sty;
   F1 – BFP based on D1;

2) $D2 = 1.089 - 0.0179 \times SFT_s$; $F2 = (4.201 / D2 - 3.813) \times 100$ [1]
   SFT_s – skin fold thickness over the scapula;
   D2 – specific body density based on SFTs;
   F2 – BFP based on D2;

3) BMI is calculated using the following formula:
   $BMI = \text{weight (kg)} / [\text{height (m)}]^2$
   $F3 = 1.2 \times BMI + 0.23 \times \text{years} - 10.8 \times \text{sex} - 5.4$
   (male = 1; female = 0) [14]
   F3 – BFP based on BMI;

The measurement of BFP using the bioimpedance method was carried out with the OMRON BF 302 instrument, which performs measurements on the upper body. Before measurements were taken, data on a patient's body height, body weight, age, and sex were entered. The device is held with extended arms at an angle of 90° in relation to the body. The elbows are held straight, and the body is not moved during the measurement. The ring finger and little finger are laid around the lower part of the electrode and the middle finger around the dents on the holder between the electrodes. With the thumb and forefinger, a patient firmly tightens the upper part of the electrode.

After taking the right position, a patient tightens the electrodes firmly with hands. The measurement takes about 20 seconds. The BFP value is seen on the display of the device. To each patient it was precisely explained how to stand and to hold the device properly. All the patients were informed about the nature of the study and were asked to sign a written consent form. They had the opportunity to end the monitoring at any time. The authors also followed the latest version of the Declaration of Helsinki given by the World Medical Association and the study was done in accordance with standards of the institutional committee on ethics (Ethics Committee of the Public Health Institute, Niš; No. 12-3785/5).

Statistical methods

The primary data were analyzed by descriptive statistical methods, methods for testing the difference of mean values, and the method for determining the correlation between variables. From the descriptive statistical methods, the measure of central tendency (mean) and measurement of variability (standard deviation) were used. To test the difference in numerical data, Student's t-test and ANOVA repeated measurements were used with the Bonferroni *post hoc* analysis. For the correlation of the tested values, the Spearman's coefficient of correlation was used. Statistical hypotheses were tested at a significance level of 0.05.

RESULTS

There were 279 participants included in the research [159 (57%) females and 120 (43%) males]. The average age was 36.09 ± 14.26 years.

Men had higher body mass and body height than women. Concerning anthropometric indexes, women had higher BFP than men (Table 1).

Using one-way ANOVA for repeated measurements, we determined a statistically significant difference between the mean values of fat percentage obtained by bioimpedance and three anthropometric methods [$F(24.19)$, $p < 0.05$]. By a further *post hoc* analysis, we found that there was a statistically significant difference between the percentage of fat determined by bioimpedance and indexes F1 and F2. There was no statistically significant difference between the values of F1 and F2. Also, there were no statistically significant differences between the percentage of fat determined by bioimpedance and index F3 (Table 2).

All the correlation coefficients between the BFP obtained by bioimpedance and other measurements by indexes F1, F2, and F3 were positive and significant. The strongest correlation was between index F3 and bioimpedance in both sexes (Table 3).

Table 1. Anthropometric indicators of examinees related to the sex (mean value ± standard deviation)

Characteristics	Whole sample (n = 279)	Men (n = 120)	Women (n = 159)	t	p
Body mass (kg)	88. 65 ± 15.96	96.37 ± 13.80	82.89 ± 15.03	- 8.311	< 0.05
Body height (m)	1.68 ± 0.1	1.75 ± 0.09	1.63 ± 0.07	- 12.48	< 0.05
BMI	31.35 ± 4.54	31.68 ±3.76	31.1 ± 5.06	-1.54	0.297
Bio (%)	31.78 ± 7.57	28.84 ±7.01	33.99 ± 7.23	11.05	< 0.05
F1 (%)	39.06 ± 26.59	32.23 ± 20.76	44.22 ± 29.28	4.09	< 0.05
F2 (%)	41.44 ± 23.91	40.22 ± 23.59	42.36 ± 24.19	0.52	0.433
F3 (%)	32.88 ± 9.04	29.5 ± 6.26	35.58 ± 8.42	12.88	< 0.05

BMI – body mass index; Bio – percentage of fat determined by bioimpedance; F1 – percentage of fat in the body determined based on SFT over the triceps; F2 – percentage of fat in the body determined based on SFT over the scapula; F3 – percentage of fat in the body based on body mass index

Table 2. Difference between mean values of the body fat percentage based on bioimpedance (Bio) and those based on the anthropometric indicators

Method	Method	p
Bio	F1	< 0.05
	F2	< 0.05
	F3	0.09
F1	F2	0.34
	F3	< 0.05
F2	F3	< 0.05

One-way ANOVA for repeated measurements, *post hoc* Bonferroni method;
F1 – percentage of fat in the body determined based on SFT over the triceps;
F2 – percentage of fat in the body determined based on SFT over the scapula;
F3 – percentage of fat in the body based on body mass index

Table 3. Correlation (Spearman–Brown correlation coefficient) between body fat percentage based on bioimpedance and anthropometric indicators in relation to sex

Method	Whole sample (n = 279)	Men (n = 120)	Women (n = 159)
F1	0.658*	0.654*	0.659*
F2	0.642*	0.638*	0.646*
F3	0.701*	0.682*	0.726*

F1 – percentage of fat in the body determined based on SFT over the triceps;
F2 – percentage of fat in the body determined based on SFT over the scapula;
F3 – percentage of fat in the body based on body mass index;
*a value of p < 0.05 was considered statistically significant

The correlation analysis in relation to age showed that all BFP determined by bioimpedance and anthropometrics were significantly and positively related. At the age of 18–25 years, the strongest correlation is between the BFP determined by bioimpedance and the F1 index (BFP based on SFT over triceps). In all other age groups, the strongest correlation was between BFP based on bioimpedance and BMI (Table 4).

Correlation analysis stratified in relation to BMI showed a significant positive correlation between the BFP based on bioimpedance and three used indexes with the exception of the F2 index for BMI ≥ 35 (our measurement of skin thickness may not have been precise enough due to the large amount of fat tissue above the scapula). In the group of the examinees whose BMI is in the range 30–34.9, the strongest correlation was between BFP based on bioimpedance and the F1 index. However, this connection is weak. In the other two groups, the correlation of BFP based on bioimpedance and the F3 index is the strongest, and this is a strong association (Table 5).

Table 4. Correlation (Spearman–Brown correlation coefficient) between body fat percentage based on bioimpedance and anthropometric indicators in relation to age

Method	Age			
	18–25	26–35	36–45	≥ 46
F1	0.676*	0.710*	0.419*	0.667*
F2	0.615*	0.631*	0.433*	0.676*
F3	0.429*	0.851*	0.618*	0.731*

F1 – percentage of fat in the body determined based on SFT over the triceps; F2 – percentage of fat in the body determined based on SFT over the scapula; F3 – percentage of fat in the body based on body mass index;
*a value of p < 0.05 was considered statistically significant

Table 5. Correlation (Spearman–Brown correlation coefficient) between body fat percentage based on bioimpedance and anthropometric indicators in relation to body mass index

Method	BMI		
	25–29.9	30–34.9	≥ 35
F1	0.558*	0.391*	0.541*
F2	0.465*	0.272*	0.222
F3	0.610*	0.285*	0.676*

F1 – percent of fats in the body determined based on SFT over the triceps;
F2 – percentage of fat in the body determined based on SFT over the scapula;
F3 – percentage of fat in the body based on body mass index; BMI – body mass index;
*a value of p < 0.05 was considered statistically significant

DISCUSSION

In our research, we show that the most appropriate anthropometric method for BFP measurement is based on BMI, because it gives the closest results and it correlates best with the modern bioimpedance method.

Today, in clinical practice and in scientific research, BMI and different indexes for determining BFP are used, but the World Health Organization officially recommends only BMI as the anthropometric method of BFP determination [15]. Some countries have developed their own standards N1, N2 [16, 17, 18]. However, there are shortcomings of this method that have been proven in various studies [19, 20, 21]. That is why there is a need to use some other anthropometric method of BFP determination, together with BMI. However, there is a problem in how to choose the appropriate index. The practice that has been proven as successful is that each country should determine the combination of indexes for BFP. It seems that body fat distribution may be country- or nation-specific [17, 22]. In our research, we compared different anthropometric indicators and, to our knowledge, the results presented here are the first of their kind in Serbia.

From all indexes which follow the percentage of fat in the body the highest mean value in the sample was determined using index based on SFT_s, whereas the lowest percentage of fat was determined using the bioimpedance method, and this method showed the lowest standard deviation. It indicates that this index was the most stable throughout the entire research. However, the method based on BMI has also a small standard deviation, which is also in favor of its

stability throughout the measurements. These results are similar to the findings of previous studies that showed that the calculation of BFP based on SFT was error-prone and with considerable variation across age, sex, and ethnicity [23]. High standard deviations with indexes based on SFT_s and b on SFT_t speak about the insufficient precision of the method.

Earlier research demonstrated a good correlation between BMI and BFP calculated or measured by different methods [24]. Nevertheless, some inconsistencies were found, most likely due to the fact that the calculation of BMI does not include age and sex. However, BFP based on BMI in our study takes into account sex and age [25, 26].

Due to this, it is highly expected that the strong correlation between the results of BFP measurer using bioimpedance and index based on BMI was found in the whole sample but also according to sex and in different age and BMI categories.

That is why the method of determining BFP using BMI can be recommended in both epidemiological studies and clinical practice. This is important since there is limited access to the advanced methods of BFP measuring in Serbia

CONCLUSION

The only anthropometric method of BFP measurement suitable for clinical practice and research is the one based on BMI because its results strongly correlate with the results based on the bioimpedance method. Anthropometric methods based on SFT over the triceps and the scapula significantly vary in the results from the method of bioimpedance and they are of low precision.

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Упоредна анализа одређивања процента масти у телу антропометријским методама и биоимпеданцом

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САЖЕТАК

Увод/Циљ Проценат масти у телу је најпоузданији показатељ степена ухрањености. За клиничку праксу важно је али и недовољно испитано да ли се у одређивању процента телесне масти треба ослањати искључиво на најсавременије методе или су поуздане и класичне антропометријске методе.

Циљ истраживања био је да се испита корелација између резултата мерења процента масти у телу савременом методом биоелектричне импеданце и класичним антропометријским методама дебљине кожног набора (ДКН) и индекса телесне масе (ИТМ).

Методе У истраживање је укључено 279 пацијената Саветовалишта за дијететику у Институту за јавно здравље Ниш током 2015. године. Проценат телесне масти класичним антропометријским мерењима одређен је на три начина: на основу ДКН над трицепсом; на основу ДКН над скапулом и на основу ИТМ. Такође свим испитаницима је апаратом ОМРОН БФ 302 на бази биоимпеданце одређен проценат телесне масти.

Резултати Једнофакторском анализом варијансе поновљених мерења утврђена је статистички значајна разлика између средњих вредности процента масти добијених биоимпеданцом и помоћу три антропометријске методе (F (24,19), $p < 0,05$). Даљом *post hoc* анализом утврдили смо да постоји статистички значајна разлика између процента масти одређеног биоимпеданцом и на основу ДКН над трицепсом и над скапулом, док антропометријска метода на основу ИТМ даје резултате сличне резултатима биоимпеданце.

Закључак У нашем истраживању показали смо да је за одређивање процента масти најпрепоручљивија антропометријска метода она на основу ИТМ, јер најбоље корелира са савременом методом биоимпеданце.

Кључне речи: проценат масног ткива; индекс телесне масе; биоимпеданца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Translation and psychometric performance of the Serbian version of the Sarcopenia Quality of Life (SarQoL®) questionnaire

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SUMMARY

Introduction/Objective The Sarcopenia Quality of Life (SarQoL®) questionnaire is a patient-reported outcome measure specific to sarcopenia.
The objective was to translate the SarQoL® questionnaire from English into Serbian and to investigate its psychometric performance.

Methods A five-stage forward-backward methodology with pre-test was used to translate the questionnaire. The validation sample in this study consisted of elderly, community-dwelling volunteers of both sexes. Three methods were used to screen for and diagnose sarcopenia: the SARC-F questionnaire (high/low risk), low handgrip strength [probable sarcopenia in the European Working Group on Sarcopenia in Older People (EWGSOP2) algorithm], and the complete EWGSOP2 criteria. We investigated the questionnaire's discriminative power, internal consistency, construct validity, and floor and ceiling effects.

Results The SarQoL® questionnaire was translated into Serbian. The validation study included 699 participants. In total, 200 participants were considered to be at high risk of sarcopenia by the SARC-F, 84 were diagnosed with low handgrip strength and 12 were confirmed to be sarcopenic. We did not find significantly lower overall QoL scores using the EWGSOP2 criteria (60.31 *vs.* 64.60; $p = 0.155$). We did find lower scores for the probably sarcopenic group (52.80 *vs.* 65.50; $p < 0.001$) and the high-risk group (50.91 *vs.* 69.02; $p < 0.001$). The Cronbach's α coefficient was 0.87, indicating a high internal consistency. Construct validity was adequate, with 75% of hypotheses on expected correlations with the SF-36 and EQ-5D questionnaires confirmed. No floor or ceiling effects were observed.

Conclusion We successfully translated the SarQoL® into Serbian, and showed that it is a valid tool for measuring QoL in the community-dwelling elderly.

Keywords: sarcopenia; quality of life; SarQoL, validation

INTRODUCTION

In 2010 the European Working Group on Sarcopenia in Older People (EWGSOP) presented its consensus definition for sarcopenia [1], and in 2019, they revised their criteria (EWGSOP2) and stated that, "Sarcopenia is a progressive and generalized skeletal muscle disorder that is associated with increased likelihood of adverse outcomes including falls, fractures, physical disability and mortality" [2]. Sarcopenia is associated with morbidity and mortality from linked physical disability, as well as with adverse outcomes including frailty, falls and fractures, poor quality of life, depression and hospitalization [3]. In the European context, the EWGSOP2 (which updates the 2010 EWGSOP criteria) are the most widely accepted. Reported prevalence rates of sarcopenia vary greatly due to differing definitions, tools of diagnosis, and patient populations [4]. Globally, the population share aged 65 years or over increased from 6% in 1990 to 9% in 2019. That proportion is projected to rise further to 16% by 2050, so that one in six people in the world will be aged 65 years or over [5, 6]. Even with a conservative estimate of prevalence, sarcopenia affects more than 50 million people today and will affect over 200 million in the next 40 years [2]. Serbia has one of the largest elderly population segments in the world. The population share of aged 65 and over is 19.4%, while the aging index (population aged 60 years and over as a proportion of those aged 0–19 years) equaled 114.3 % [7, 8].

Until 2015, researchers only had generic questionnaires, such as the SF-36, available to assess the quality of life of sarcopenic patients. These questionnaires are designed for use in broad populations and may thus not be sensitive enough to accurately measure the quality of life in sarcopenic populations [9]. To address this problem, Beaudart et al. [10] developed the Sarcopenia Quality of Life (SarQoL®) questionnaire. The SarQoL® questionnaire is a non-diagnostic instrument but a patient-reported outcome measure specific to sarcopenia.

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The SarQoL® questionnaire consists of 22 questions incorporating 55 items, which fall into seven domains of health-related quality of life. These domains are "Physical and Mental Health," "Locomotion," "Body Composition," "Functionality," "Activities of Daily Living," "Leisure activities," and "Fears". Each domain is scored from 0 to 100, and an Overall QoL score is calculated. The questionnaire is auto-administered and takes 10 min to complete [10]. The questionnaire is available in 30 languages and can be found on its webpage [11]. The psychometric properties of the SarQoL® have already been demonstrated [12] and it has been validated for several languages such as English [13], Romanian [14], Hungarian [15], Polish [16], Greek [17], Dutch [18], Spanish [19], Lithuanian [20] and Russian [21], but so far, the SarQoL® was not available in Serbian.

To ensure the usability and cultural suitability of the questionnaire, it is necessary to involve the target population in the translation process, with the aim of maximizing compatibility, improving quality and completeness and adaptation to cultural differences. The objective of this study was to translate the SarQoL® questionnaire into Serbian language and to investigate the discriminative power, construct validity, internal consistency and presence of floor or ceiling effects.

METHODS

The translation of the SarQoL® questionnaire into Serbian was performed according to the translation guidelines formulated by Beaton et al. [22]. Five different phases were followed. First, there were two initial independent translations from English into Serbian by professional translators, both Serbian native speakers. In phase two, the synthesis of the two translations was done to provide a single "first version" of the translated questionnaire. Next phase included the backward translation by two independent translators, unfamiliar with the original English version. The expert committee was established, and it included four translators, one Serbian and one English linguist. They reviewed and compared the back translations with the original questionnaire and consent was given for the "second version" of the translated questionnaire in phase four. In the last phase, the "second version" of the questionnaire was administered to 25, older, community-dwelling subjects from both genders, who afterwards gave their feedback about the comprehensibility of and the language used in the questionnaire as well as any cultural issues present in the questionnaire's questions. That information was included in the "final version" of the Serbian SarQoL®.

The sample in this study consisted of community dwelling volunteers of both sexes, recruited through Pensioners' association of Novi Sad, Serbia, from March to June 2019. Inclusion criteria were 65 years of age or older, native Serbian speaker, and able to understand and complete the study related questionnaires. Participants were excluded if they were immobilized, had an amputated limb, suffered from an unstable chronic and/or severe medical disease, or from any neuropsychiatric disorder that could influence their collaboration. All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

We used three different ways to screen and diagnose sarcopenia. SARC-F questionnaire was used for quick screening and rapid diagnosis, hand grip strength (HGS) has been used as important index of low muscle strength and EWGSOP2 criteria were used to diagnose sarcopenia.

Osteodensitometry provided data of appendicular skeletal mass, representing the sum of lean mass at upper and lower limbs and when divided with height squared, is used to obtain skeletal muscle index. Patients were considered to have low muscle mass when the appendicular skeletal mass was < 15 kg in women and < 20 kg in men, or the skeletal muscle index was < 5.5 kg/m^2 in women and < 7.0 kg/m^2 in men [2]. In this study we used GE Healthcare Lunar iDXA (GE Healthcare, Chicago, IL, USA).

Malmstrom and Morley [23] developed a questionnaire called SARC-F, simple, secure and inexpensive screening tools with good performance convenient and helpful to the medical staff to screen patients for sarcopenia. The questionnaire comprises of five questions about strength, assistance in walking, rising from a chair, climbing stair and falls. Each component is given 0–2 points; a total score of the questionnaire is between 0 and 10 points, with score ≥ 4 points is reported to be predictive of sarcopenia [3]. Previous investigations on the diagnostic accuracy of the SARC-F questionnaire for sarcopenia diagnosed with the EWGSOP2 criteria have shown that this tool possesses moderate to high sensitivity and specificity. A meta-analysis based on four studies found a sensitivity of 77% (95% CI: 49–92%) and a specificity of 63% (95% CI: 43–79%) [24].

The Sammons Preston Jamar hydraulic hand dynamometer (Patterson Companies, Patterson Medical Supply Inc., St Paul, MN, USA) was used as method for muscle strength measurement. Participants were seated in a standard chair, six measures were taken, tree with each arm and the highest result of both hands is reported as the final value [25]. Low muscle strength was defined as grip strength values of < 16 kg for women and < 27 kg for men [2].

Physical performance was evaluated with usual gait speed on a four-meter track as part of Short Physical Performance Battery test. Values under ≤ 0.8 m/s of gait speed were used as the threshold for identifying low gait speed and poor physical performance [2].

Besides the SarQoL-Srb®, two other questionnaires were administrated to the population, the SF-36 questionnaire and The European Quality of Life 5-Dimension-3 Level questionnaire.

We validated the psychometric properties of the SarQoL-Srb® by assessing its discriminative power, internal consistency, and potential floor and ceiling effects, followed by determination of the construct validity according to recommendations proposed by Terwee et al. [26].

The SarQoL® questionnaire is an instrument designed specifically for the purposes of the sarcopenic population

with its discriminative power, and as such should have the ability to differentiate between sarcopenic and nonsarcopenic subjects on the overall quality of life score. Internal consistency, a measure of the questionnaire's homogeneity, was assessed with the Cronbach's α coefficient. By deleting one domain at a time, each domain's impact on the internal consistency of the questionnaire was also considered. The correlation of each domain with the total score of the SarQoL-Srb® was also assessed using Pearson's correlations, and using Pearson's or Spearman's correlations in function of the score distributions [27]. The construct validity examines whether the questionnaire really measures the construct it claims to measure. We evaluated hypotheses on the expected correlations between SarQoL® and similar or different domains of the other two questionnaires. For the convergent validity, the hypotheses for this study are that strong correlations will be found between the Overall score of the Serbian SarQoL® questionnaire and the domains "Physical Functioning," "Vitality," and "Role Limitation due to Physical Problems" of the SF-36; as well as between the Overall score of the Serbian SarQoL® questionnaire and the Utility Index of the EQ-5D. Divergent validity examines correlations between the SarQoL® questionnaire and domains of other questionnaires that should, in theory, be different. The hypotheses are that weak correlations will be found between the Overall score of the Serbian SarQoL® questionnaire and the domains "Mental Health" and "Role Limitation due to Emotional Problems" of the SF-36 questionnaire. We also expected to find weak correlations between the Overall score of the Serbian SarQoL® questionnaire and the questions related to Self-Care and Anxiety/Depression of the EQ-5D. The questionnaire possesses good construct validity if at least 75% of the hypotheses are confirmed [26]. Floor and ceiling effects are observed when more than 15% of respondents obtain either the highest score (ceiling effect) or the lowest score (floor effect) possible.

All analyses described here were performed using Python 3.6 (Python Software Foundation, Wilmington, DE, USA) and R 3.0.1 (R Core Team, Vienna, Austria) programming languages, with a level of significance of $\alpha = 0.05$.

RESULTS

Initially, 700 participants were included in study, but one withdrew informed consent so 699 subjects were screened for sarcopenia. The median age was 70 (67–74) years, the sample consisted of 191 male participants (27.3%), and 508 were female (72.7%). A total of 12 patients were diagnosed as sarcopenic as they fulfilled the criteria for sarcopenia according to the EWGSOP2 definition.

In our study group, sarcopenic subjects were significantly older and had a lower BMI compared to non-sarcopenic individuals 76 (72–80.25) *vs.* 70 (67–74) years ($p = 0.004$) and 29.55 (26.25–32.55) *vs.* 26.60 (24.83–28.84) kg/m^2 ($p = 0.014$). The complete clinical characteristics are shown in Table 1.

Table 1. Descriptive data of the sample

Parameters	All (n = 699)	No sarcopenia (n = 687)	Sarcopenia (n = 12)	p-value
Age (years)	70 (67–74)	70 (67–74)	76 (72–80.25)	0.004
Gender				0.855
Women	508 (72.7)	499 (72.6)	9 (75)	
Men	191 (27.3)	188 (27.4)	3 (25)	
Marital formal status				0.051
Single	43 (6.2)	43 (6.3)	0 (0)	
Married	356 (51.4)	354 (51.5)	2 (16.7)	
Relationship	17 (2.4)	17 (2.5)	0 (0)	
Divorced	53 (7.6)	51 (7.4)	2 (16.7)	
Widowed	230 (32.9)	222 (32.3)	8 (66.7)	
Educational status				0.861
Primary education (4 years)	26 (3.7)	26 (3.8)	0 (0)	
Elementary education (8 years)	155 (22.2)	153 (22.3)	2 (16.7)	
Secondary education	355 (50.8)	349 (50.8)	6 (50)	
Higher education	144 (20.6)	140 (20.4)	4 (33.3)	
Master's degree	9 (1.3)	9 (1.3)	0 (0)	
PhD	10 (1.4)	10 (1.5)	0 (0)	
Smoker				0.882
No	632 (90.4)	621 (90.4)	11 (91.7)	
Yes	67 (9.6)	66 (9.6)	1 (8.3)	
Body Mass Index	29.41 (26.2–32.38)	29.55 (26.25–32.55)	26.60 (24.83–28.84)	0.014
Mini-Mental State Exam	29 (26–29.75)	29 (26–30)	28 (26–29)	0.326
Gait speed	0.83 ± 0.24	0.83 ± 0.24	0.60 ± 0.26	0.081
Grip strength	24.5 (19–30)	25 (20–31)	12 (10–14)	< 0.001
Appendicular skeletal mass	18.67 (16.25–22.02)	18.70 (16.38–22.18)	14.38 (13.19–14.93)	< 0.001

Notes: Values are expressed as median (25–75%) for quantitative variables that did not follow a normal distribution and frequencies (percentages) for the categorical variables

Table 2. Discriminative power of the SarQoL-Srb®

Tests	EWGSOP2			SARC-F			Grip strength		
Parameters	No sarcopenia	Sarcopenia	P[a]	Low risk	High risk	P[a]	Normal	Low	P[a]
N	687	12		499	200		614	85	
D1: Physical and mental health	65.53 (54.43–79.97)	55.53 (49.43–74.13)	0.136	72.20 (59.53–85.53)	52.20 (45.26–59.62)	<0.001	66.63 (55.53–81.56)	55.53 (44.43–68.87)	< 0.001
D2: Locomotion	53.12 (47.22–68.89)	54.17 (43.75–80.56)	0.953	55.56 (50–72.22)	50 (44.27–58.33)	<0.001	53.87 (47.22–63.89)	50 (44.44–61.11)	0.013
D3: Body composition	66.67 (54.17–79.17)	58.33 (44.79–62.50)	0.104	70.83 (58.33–83.33)	54.17 (45.83–66.67)	<0.001	66.67 (54.17–79.17)	58.33 (50–75)	< 0.001
D4: Functionality	71.15 (57.69–83.01)	63.46 (43.44–62.37)	0.136	77.08 (67.31–87.50)	52.08 (45.73–61.54)	<0.001	73.08 (60.49–83.93)	55.77 (46.15–73.08)	< 0.001
D5: Daily activities	61.67 (48.33–75)	55 (44.81–61.67)	0.059	66.67 (58.33–78.33)	45 (36.67–56.67)	<0.001	63.33 (51.67–75)	48.33 (38.46–61.67)	< 0.001
D6: Leisure activities	33.25 (33.25–66.50)	33.25 (29.09–54.03)	0.799	33.25 (33.25–66.50)	33.25 (16.62–33.25)	<0.001	33.25 (33.25–66.50)	33.25 (16.62–49.88)	0.009
D7: Fears	87.50 (87.50–100)	87.50 (75–100)	0.259	100 (87.50–100)	87.50 (75–87.50)	<0.001	87.50 (87.50–100)	87.50 (75–100)	< 0.001
Overall score	64.60 (54.93–74.50)	60.31 (44.48–68.85)	0.155	69.02 (61.94–77.98)	50.91 (44.76–57.01)	<0.001	65.50 (56.01–75.25)	52.80 (45.71–66.17)	< 0.001

[a]All p-values were obtained with Mann–Whitney U-test for independent samples

Discriminative power

Sarcopenic subjects by EWGSOP2 have reported slightly lower global quality of life scores compared to non-sarcopenic subjects (60.31 (44.48–68.85) *vs.* 64.60 (54.93–74.50), p = 0.155). The domains of physical and mental health, locomotion, functionality and daily activities were also scored lower in sarcopenic subjects compared to non-sarcopenic ones (Table 2).

SARC-F divided the study group to 200 subjects (28.6%) who were at high risk of sarcopenia, and their quality of life was significantly reduced in SarQoL® (69.02 (61.94–77.98) *vs.* 50.91 (44.76–57.01) p < 0.001) and they scored significantly lower in all domains when compared to subjects with low risk for sarcopenia (Table 2).

When the sample was divided into those with low grip strength versus those with normal grip strength, we had 85 subjects (12.1%) with low grip strength. They had significantly lower global quality of life in SarQoL® (65.5 (56.01–75.25) *vs.* 52.8 (45.71–66.17) p < 0.001) and all domains were scored significantly lower comparing to group with normal grip strength (Table 2).

Internal consistency

The Cronbach's α of the Serbian version of the SarQoL® was 0.87, indicating a high internal consistency. Deleting the domains one at the time, led to Cronbach's α values varying between 0.83 (when deleting the domain four "Functionality") and 0.89 (for the domain six "Leisure activities"). When comparing each domain with the SarQoL® total score, a significant positive correlation for all domains was observed with values ranging from good (0.41, D6 – leisure activities) to excellent (0.92, D4 – functionality) as shown in Table 3.

Construct validity

The results of construct validity are available in Table 4. As expected, strong/good correlations were found between the SarQoL® and some domains of the SF-36 questionnaire which were supposed to have similar dimensions such as physical functioning (SF-36-PF and role limitation due to physical problems (SF-36-RLPP as well as with the utility score of the EQ-5D questionnaire) and the questions of the EQ-5D questionnaire related to mobility and usual activities. There are four hypotheses for convergent validity, each being that there are moderate to strong correlations

Table 3. Intercorrelations between the Serbian SarQoL® questionnaire total and domains scores (n = 699)

Activities	D1	D2	D3	D4	D5	D6	D7	Overall
	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho
D1	1							
D2	0.43*	1						
D3	0.69	0.34*	1					
D4	0.75*	0.42*	0.58*	1				
D5	0.7*	0.41*	0.53*	0.77*	1			
D6	0.29*	0.11*	0.27*	0.37*	0.34*	1		
D7	0.54*	0.29*	0.41*	0.55*	0.50*	0.27*	1	
Overall	0.85*	0.57*	0.68	0.92*	0.90*	0.41*	0.60*	1

*Correlation is significant at the 0.05 level (two tailed);
D1 – physical and mental health; D2 – locomotion; D3 – body composition; D4 – functionality; D5 – daily activities; D6 – Leisure activities; D7 – Fears

Table 4. Construct validity (n = 699)

Convergent validity	Rho	p
SF-36 Physical functioning	0.760	0.002
SF-36 Role limitation due to physical problems	0.637	0.001
SF-36 Vitality	0.656	0.005
EQ-5D Index score	0.589	< 0.001
Divergent validity		
SF-36 Role limitation due to emotional problems	0.490	< 0.001
SF-36 Mental health	0.474	< 0.001
EQ-5D Anxiety	-0.332	< 0.001
EQ-5D Self care	-0.332	< 0.001

expected between SarQoL overall score and SF-36-PF, SF-36-VIT, SF-36-RLPP and the EQ-5D-UI. All of the mentioned correlations are strong as is shown by the values in Table 4. The hypotheses for divergent validity claim that we expect weak or no correlations between SarQol Overall score and SF-36-MH, SF-36RLEP, EQ-5D-SC and EQ-5D-AD. Out of these four correlations, two are relatively weak, with the two EQ-5D items, and the remaining two (MH and RLEP) show moderate strength in the correlation. The two positive correlations go against the hypotheses, leaving 75% of the hypotheses confirmed, which is incidentally the cut-off used to evaluate construct validity. Considering these results, we can conclude that SarQol-Srb® has had its construct validity confirmed.

Floor and ceiling effects

No subjects presented with the lowest score to the questionnaire (0 points) or the maximum score (100 points) on the overall QoL score of the Serbian SarQoL® questionnaire. Therefore, neither floor or ceiling effects were found for the questionnaire.

DISCUSSION

The SarQoL® is the first quality of life questionnaire specifically developed for sarcopenia. The present study was conducted following a standardized validation protocol as advised by the creators of the original SarQoL® questionnaire. The transcultural adaptation resulted in a valid Serbian version, psychometrically matched with the original version. Its high internal consistency, and construct validity certifies the measurement quality of the translated version. A meticulous methodology was used, providing protection against subjectivity in the translation and assuring equivalence between the original English SarQoL® questionnaire and the Serbian translation. The time required to complete the Serbian SarQoL® was between 10 and 20 minutes, longer than the time reported by Beaudart et al. [13].

We enlisted a reasonably adequate cohort of older individuals for screening hoping to collect a representative sample of people with sarcopenia but out of 699 subjects, we had only 12 with sarcopenia as diagnosed with the EWGSOP2 criteria. Our hypothesis to explain very low prevalence of sarcopenia is that those who applied to participate in the study may be from a more physically and psychologically active group who tend to also take part in activities like hiking, dancing and other social events, organized medical check-ups, in their pensioners' association. This means that the study participant selection may have introduced a bias and focused on the healthier section of population. In our study group, sarcopenic subjects were significantly older and had a lower BMI compared to non-sarcopenic individuals and there was a higher proportion of widows/widowers in the sarcopenic group.

In the analysis of discriminative power, when HGS and SARC-F questionnaire were used, all domains and overall quality of life had lower results with significant p-values. Our analysis showed that when the EWGSOP2 criteria were applied, sarcopenic subjects reported a slightly lower global quality of life compared to non-sarcopenic subjects in the Serbian SarQoL® total score. The domains of D1-physical and mental health, D3-body composition, D4-functionality and D5- activities of daily living were also lower scored in sarcopenic subjects compared to non-sarcopenic ones. We found no differences for D2-locomotion, same as Fábrega-Cuadros et al. [19] in the Spanish SarQoL® validation, in D6 – Leisure activities as Gasparik et al. [14] in the Romanian validation, and also in D7-Fears. Differences found when sample was divided on a basis of HGS and SARC-F might be due to larger groups. We had just 12 subjects with all EWGSOP2 criteria in regard to 200 subjects with high risk for sarcopenia based on SARC-F score and 85 with low hand grip. The diagnostic performance of the SARC-F in this sample is not in line with what has previously been reported about its sensitivity and specificity. We do not have an explanation for this phenomenon in this specific sample, and we think that a diagnostic accuracy study of the SARC-F in a Serbian population should be performed in the near future [28].

The Cronbach's α coefficient of the Serbian version of the SarQoL® was 0.87, indicating a high internal consistency. Deleting the domains one at the time, led to Cronbach's α values varying between 0.83 (when deleting the domain 4 "Functionality") and 0.89 (for the domain 6 "Leisure activities"). When comparing each domain with the SarQoL® total score), a significant positive correlation for all domains was observed with values ranging from good (0.41, D6 – leisure activities) to excellent (0.92, D4 – functionality). The specificity of the Serbian version of the SarQol survey is that it shows a strong positive correlation of the domain D7-Fears. The correlation can be attributed to the unstable political and economic environment which has led to a fall in the quality of social and health services provided. The fall is especially prevalent in the support given by health workers in helping those who have lost their independence in daily life activities.

As expected, strong/good correlations were found between the Serbian version of the SarQoL® and some domains of the SF-36 questionnaire as with the utility score of the EQ-5D. We found weaker correlations between domains of the Serbian version of the SarQoL® which were supposed to have different dimensions. These results are in congruence with those reported in other studies [9, 13, 14].

This study has some limitations. Firstly, our sample only comprises 12 sarcopenic subjects (1.7%) which is much lower comparing to other studies and thus this population does not reflect exactly a sarcopenic population [12, 13, 20, 27]. Our study participant selection may have introduced a bias and focused on the healthier section of population by relying on volunteers despite the known fact that sarcopenic individuals are less likely to volunteer for clinical studies due to their physical difficulties [4]. The sample that was recruited for this study was not a random sample and should be complemented with participants from nursing homes or elderly more dependent on their care providers. Another limitation of this study is due to the issues related to the lack of a test-retest reliability evaluation. However,

test-retest reliability at a two-week interval has shown to be excellent in the other validation of the SarQoL® and should therefore not be an issue [13, 18].

CONCLUSION

In conclusion, the results of the present study confirm that, the Serbian SarQoL® can discriminate with significant p-values between older adults with and without sarcopenia if the HGS and SARC-F are used, but we were unable to confirm discriminative power when using the EWGSOP2 criteria in this sample. Also, in Serbian population aged 65 and over, the Serbian version of the SarQoL® shows high internal consistency, as well as good convergent and divergent validity for a sarcopenic population.

Conflict of interest: None declared.

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Превод и психометријске перформансе српске верзије упитника „Квалитет живота са саркопенијом“ (*Sarqol®*)

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САЖЕТАК

Увод/Циљ Упитник „Квалитет живота са саркопенијом“ (*SarQoL®*) који попуњавају болесници специфичан је за саркопенију.

Циљ је био да се преведе упитник са енглеског на српски језик и испитају његове психометријске перформансе.

Методе Упитник је преведен. Испитаници су били пензионери оба пола, старији од 65 година. Три методе су коришћене за утврђивање саркопеније: упитник *SARC-F*, смањена снага стиска и комплетни критеријуми Европске радне групе за саркопенију код старијих особа (*EWGSOP2*). Испитивани су дискриминативна способност, интерна конзистенција, конструкциона валидност, ефекат пода и плафона.

Резултати Упитник *SarQoL®* је преведен на српски језик. Валидациона студија је спроведена на 699 испитаника. Од укупног броја, 200 учесника припадају високоризичној групи за саркопенију на основу упитника *SARC-F*, 84 испитаника је имало ослабљену снагу стиска шаке, а 12 потврђену саркопенију. На основу критеријума *EWGSOP2* није установљен значајно мањи квалитет живота (60,31 *vs*. 64,60; $p = 0,155$). Добијене су ниже вредности упитника *SarQoL®* за групу са мањим ризиком за саркопенију (52,80 *vs.* 65,50; $p < 0,001$) и високоризичну групу за саркопенију (50,91 *vs*. 69,02; $p < 0,001$). Кронбахов алфа коефицијент је износио 0,87, што указује на високу интерну конзистенцију. Конструктивна валидност је била адекватна и потврђена са 75% и очекиваном корелацијом са упитницима *SF-36* и *EQ-5D*. Није уочен ефекат пода/плафона у добијеним резултатима.

Закључак Упитник *SarQoL®* је успешно преведен на српски језик и потврђена је његова валидност за утврђивање квалитета живота геријатријске популације.

Кључне речи: саркопенија; квалитет живота; *SarQoL*, валидација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A case of primary hepatic lymphoma and a review of literature

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SUMMARY

Introduction Primary hepatic lymphoma (PHL) is a rare disease and represents lymphoproliferative disorder confined to the liver parenchyma. This condition is difficult to distinguish from other liver diseases. Histopathology is essential and confirms the diagnosis. Treatment options for PHL include surgery, chemotherapy, radiation, or combinations of these modalities. The objective of this this report is to present a case of PHL and to discuss disease features and treatment options in order to facilitate diagnostics and therapy.

Case outline A 72-year-old female was presented with abdominal pain, nausea, weight loss, and fatigue. Computed tomography (CT) revealed hypoattenuating solitary lesion in liver segment VIII. The detected liver lesion showed elevated 2-[fluorine 18] fluoro-2-deoxy-D-glucose uptake on positron emission tomography/CT examination. Extrahepatic disease was not detected. The tumor board opted for surgery, and atypical resection of liver segments VII and VIII was performed. Pathohistological examination of the resected tumor demonstrated liver infiltration with non-Hodgkin's lymphoma, diffuse large B-cell type. Postoperative recovery was complicated by fluid collections in the right subphrenic space, successfully managed by percutaneous drainage. Subsequently, the patient was treated with chemotherapy and attained a complete remission documented by negative CT findings.

Conclusion PHL can easily be misdiagnosed as another more frequent primary liver tumor due to its non-specific clinical manifestations, laboratory and imaging findings, and therefore should be considered in the differential diagnosis of a hepatic lesion.

Keywords: chemotherapy; drainage; liver; operative; tumor

INTRODUCTION

Primary hepatic lymphoma (PHL) is a lymphoproliferative disorder confined to the liver without any evidence of involvement of the spleen, lymph nodes, bone marrow, or other lymphoid structures [1]. PHL is a rare disease and accounts for 0.4% of all extranodal lymphoma and 0.016% of all non-Hodgkin's lymphoma (NHL) [2]. PHL of diffuse large B-cell lymphoma (PHL-DLBCL) is more infrequent [3].

PHL typically occurs in middle-aged men, and the presenting symptoms, blood investigations, and imaging findings are usually nonspecific [1]. This condition is difficult to distinguish from primary liver cancer, liver metastases, granulomatous pseudotumor, and other liver diseases; therefore, it is easily misdiagnosed [3]. Histopathology is mandatory and confirms the diagnosis.

A patient with PHL is presented. A better understanding of the disease will facilitate diagnostics and therapy. We contribute our experience to the pool of data. Written informed consent was obtained from the patient for publication of this case report and accompanying images.

CASE REPORT

A 72-year-old female presented with abdominal pain, nausea, weight loss, and fatigue was examined in the emergency room. Abdominal ultrasound revealed an irregular heterogeneous, predominantly hypoechoic, 42 × 45 mm lesion in liver segments VII and VIII. Computed tomography (CT) revealed a 58 × 49 × 54 mm hypoattenuating lesion, adjacent to the right hepatic vein, without signs of infiltration, in segment VIII on non-contrast scans, with rim-enhancement in the arterial phase (due to mass effect on the surrounding liver parenchyma) with the same attenuation as liver parenchyma during the delayed phase.

Liver enzymes and bilirubin values were within the normal range. The serum tumor marker levels (carcinoembryonic antigen – CEA, carbohydrate antigen 19-9 – CA 19-9, and alpha-fetoprotein – AFP) were not elevated. The patient was negative for human immunodeficiency virus (HIV), hepatitis B and C (HBV, HCV), and Ebstein–Barr virus (EBV).

The patient underwent 2-[fluorine 18] fluoro-2-deoxy-D-glucose (FDG) positron emission computed tomography (^{18}F-FDG PET-CT) for further functional characterization of the detected lesions and for staging of the disease. The exam revealed elevated ^{18}F-FDG uptake

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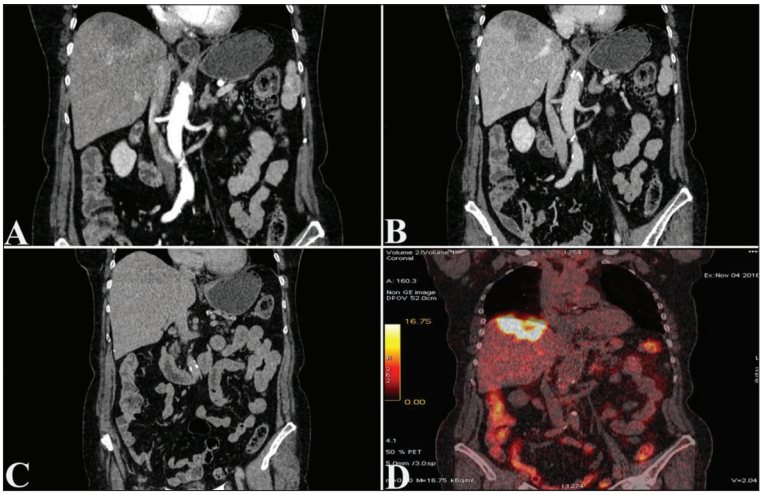

Figure 1. Contrast-enhanced abdominal computed tomography and F-18 FDG positron emission tomography scan in the coronal plane: (A) arterial phase showing a hypoattenuating, slightly lobulated, lesion in segment VIII with peripheral rim-enhancement; (B) hypodense lesion during the portal phase; (C) same attenuation as liver parenchyma during the delayed phase; (D) hypermetabolic mass lesion in the liver

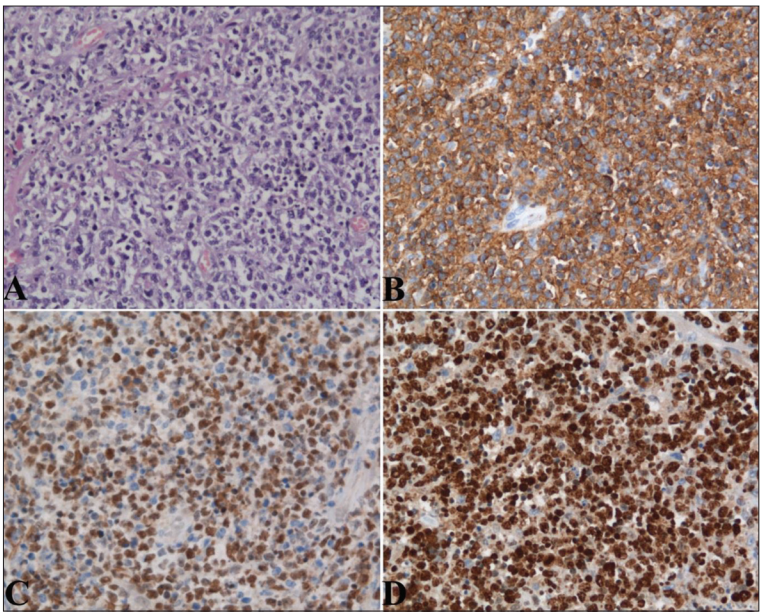

Figure 2. Microscopic findings of the liver confirming non-Hodgkin's diffuse large B-cell lymphoma; (A) diffuse proliferation of lymphoid cells (H&E, ×200); (B) positive for CD20; (C) positive for BCL6; (D) positive for Ki-67

only in liver segment VIII without pathological elevation in other organs (Figure 1). On esophagogastroduodenoscopy atrophic gastritis was present. Colonoscopy detected anorectal polyps and enlarged hemorrhoidal nodes. A barium enema study revealed diverticulosis of the descendent and sigmoid colon. Extrahepatic disease was not detected.

The multidisciplinary tumor board opted for surgery and atypical resection of liver segments VII and VIII was performed. Lymphatic nodes in the hepatoduodenal ligament were dissected for frozen section analysis and proved to be benign.

Pathohistological examination of resected tumor revealed hepatic tissue with large nodular, tumorous, lymphoid infiltrates and numerous confluent necrotic fields, without tumor cells on the resection margin.

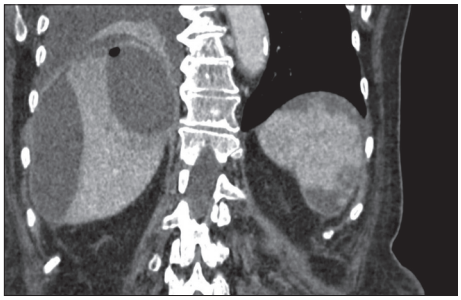

Figure 3. Postoperative computed tomography scan in the coronal plane demonstrating pleural effusion, fluid collection in the right subphrenic space, and viscous fluid collection in segment VI of the liver

Immunohistochemical staining showed that 90% of tumor cells had positive reaction for CD 20, CD79a, bcl 6, MUM 1, and Ki 67. These histological and immunohistochemical findings demonstrated liver infiltration with DLBCL (Figure 2).

Three weeks after the hospital discharge, the patient was presented with abdominal pain, nausea, and vomiting and was promptly readmitted. Routine blood and biochemistry examination revealed elevated inflammatory markers and abdominal CT demonstrated pleural effusion, 150 × 97 × 103 mm fluid collection in the right subphrenic space with a mean attenuation value of 22 HU and viscous fluid collection in segment VI of the right liver lobe, containing gas inclusions, 95 × 57 × 110 mm in size (Figure 3).

The patient was referred to the interventional radiology department for percutaneous drainage of the fluid collection at the liver resection site. Ultrasound guided initial puncture of the right subphrenic fluid collection was performed and specimen of viscous yellow fluid was sent for microbiological analysis followed by the placement of 10.2 Fr percutaneous drainage catheter in collection. Enterobacter spp. were isolated from the specimen and antibiotic therapy was initiated according to the antibiogram. Control CT scan of the abdomen revealed resolution of the subphrenic collection around the tip of the pigtail catheter. On follow-up ultrasound examination, no fluid collection was detected and the patient was referred to the department of hematology for further treatment.

Bone marrow biopsy did not reveal lymphoma infiltration. Complete blood test excluded leukemic cells in the peripheral blood. Due to reduced left ventricular function (EF 55%), the lymphoma board decided to treat the patient with a R-miniCHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, prednisone) low-dose chemotherapy protocol. She was given eight cycles of R-miniCHOP at three-week intervals. During the course

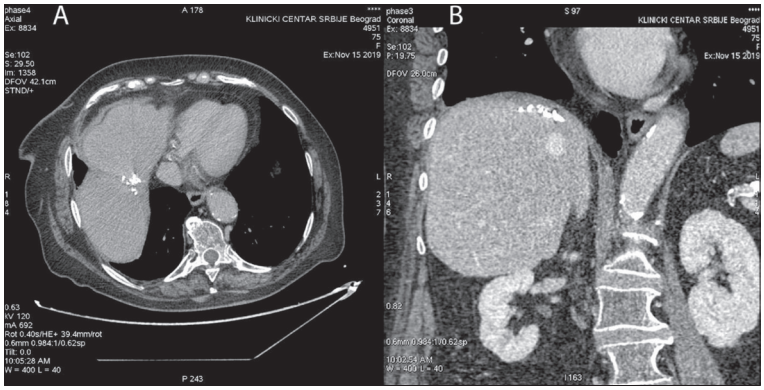

Figure 4. Computed tomography scan after a three-year follow-up in the axial and coronal planes, demonstrating no signs of recurrent disease

of chemotherapy, the patient occasionally complained of tingling in her hands and feet, so vincristine was replaced with velban from the sixth cycle to the end of treatment.

During the three-year follow-up the patient showed no symptoms or signs of recurrent disease (Figure 4).

DISCUSSION

PHL is an extra-nodal lymphoma of the liver without involvement of any other organ (lymph node, spleen, etc.) [4].

The most common presenting symptom in PHL is abdominal pain, occurring in more than 40% of patients. The B-symptoms of fever and weight loss occur in about one-third of the patients. Jaundice is seen in less than 20% of patients [5]. Our patient's clinical presentation included abdominal pain, nausea, weight loss, fatigue and were consistent with that of primary hepatic NHL.

Preexisting liver disease, such as chronic liver cirrhosis secondary to hepatitis B virus, hepatitis C virus, or hemochromatosis, is present in less than 9% of the patients [6]. In rare circumstances, progressive hepatitis and acute hepatic failure with rapid progression and poor prognosis may occur [7, 8].

The presented patient was diagnosed with NHL by pathohistological examination of the resected tumor. The pathological type was DLBCL. The involvement of other sites was excluded by lymph node examination, radiologic examinations (ultrasound, CT, PET/CT, barium enema), and endoscopic examination (esophagogastroduodenoscopy, colonoscopy).

Most PHL patients show a two- to three-fold increase in alanine aminotransferase and aspartate aminotransferase levels, with an increase in bilirubin and high-density lipoprotein levels. However, AFP and CEA levels are usually normal, in contrast to those in primary liver cancer and liver metastases [3]. The presented patient had normal liver enzyme and bilirubin values. Tumor markers (CEA, CA19-9, AFP) were not elevated.

The exact incidence and prevalence of PHL is not known. PHL is rare and comprises 0.4% of all cases of extra nodal NHL and 0.016% of all cases of NHL [2]. The male-to-female ratio is 1.9:1 [9]. The patient in this report was a 72-year-old female, although PHL is more common in males of around 50 years old.

Although PHL is rare, persistent inflammatory processes associated with HCV infection or autoimmune disease may play independent roles in the lymphomagenesis of hepatic B cells [10]. The presented patient was negative for HIV, AIDS, HBV, HCV, and EBV and did not have any autoimmune disease.

There are three radiological presentations of PHL: 1) a solitary lesion, 2) multiple lesions within the liver, and 3) diffuse hepatic infiltration. The most common presentation is a solitary lesion. The initial radiologic test is usually an ultrasound that demonstrates a large, solitary hypoechoic lesion or multiple hypoechoic lesions resembling metastasis [11]. On CT scans, PHL lesions appear as hypoattenuating lesions and may have a central area of low intensity indicating necrosis. Following the administration of intravenous contrast, 50% of PHL lesions do not enhance, 33% show patchy enhancement, and 16% show a ring of enhancement [11]. CT scan of this patient revealed a hypoattenuating lesion in liver segment VIII on non-enhanced scans, with rim-enhancement in the arterial phase, conceding with 16% ring enhancement as described in literature.

Due to the rarity of this disease entity and its nonspecific clinical presentation and laboratory and radiologic features, a definite clinical diagnosis of PHL is difficult. PHL may be confused with hepatitis, primary hepatic tumors, hepatic metastases from gastrointestinal carcinoma, and systemic lymphoma with secondary hepatic involvement [12].

Percutaneous liver biopsy is the most valuable tool for the diagnosis of PHL. The transjugular approach is an alternative when a discrete mass is not visible on imaging for percutaneous liver biopsy [13]. A liver biopsy was not done because the liver lesion appeared malignant on radiological assessment and in order to avoid the risk of tumor seeding in resectable patient. Similar scenarios are described in the literature, where patients, incorrectly diagnosed as PHL, were operated on for other diagnosis.

Treatment options for PHL include surgery, chemotherapy, radiation, or combinations of these modalities.

It has been suggested that, for low-volume localized PHL, surgical resection, alone or in combination with chemotherapy, might be a treatment of choice. Lei [6] described 10 patients treated with curative intent surgery who had a median survival of 22 months, ranging 1.5–120 months. Avlonitis and Linos [5], in a large review of the literature, showed similar results that patients treated by surgery and followed by chemotherapy have better survival rates, with median survival of 15.3 months (range: 0–123.6 months). The current indications for surgery, based on the available data from the literature, include localised disease that can be completely resected [5].

R-CHOP chemotherapy regimen is the standard treatment for patients with DLBCL. There are reported cases of complete response to R-CHOP alone in patients with PHL-DLBCL [14, 15]. Whether systemic chemotherapy alone will give results comparable to surgery in resectable cases is currently unclear [16].

In conclusion, PHL is a rare disease and can be misdiagnosed as another more frequent primary liver tumor due to its non-specific clinical presentation, laboratory and imaging findings, and therefore should be considered in the differential diagnosis of a hepatic lesion. Pathohistological analysis is essential for definite diagnosis, determining PHL subtype and the extent of involvement of surrounding tissue. There is no consensus on the optimal treatment for PHL and the prognosis is variable. All complications during treatment should be diagnosed on time and properly managed. Further prospective studies are mandatory for providing treatment guidelines.

Conflict of interest: None declared.

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Примарни лимфом јетре и преглед литературе

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САЖЕТАК

Увод Примарни лимфом јетре је ретка болест и представља лимфопролиферативни поремећај ограничен само на јетру. Ову болест је тешко разликовати од других болести јетре. Хистопатолошка верификација је од кључне важности и потврђује дијагнозу. Терапијски третман подразумева хируршко лечење, хемиотерапију, радиотерапију и комбинацију ових модалитета.

Циљ овог рада је приказ болесника са примарним лимфомом јетре и дискусија о карактеристикама болести које ће допринети њеном бољем разумевању и олакшати постављање дијагнозе и лечење.

Приказ болесника Болесница старости 72 године јавила се лекару због бола у трбуху, мучнине, губитка телесне тежине и малаксалости. Комјутеризованом томографијом откривена је солитарна хиподензна промена у VIII сегменту јетре. Описана промена је показала повећано преузимање ^{18}*F-FDG* на *PET* прегледу компјутеризованом томографијом. Није откривено присуство болести ван јетре. Онколошки конзилијум се одлучио за хируршко лечење, после чега је учињена ресекцију VII и VIII сегмента јетре. Патохистолошком анализом доказана је инфилтрација ткива јетре не-Хоџкиновим лимфомом, дифузни Б-крупноћелијски тип. Постоперативни ток је био компликован развојем течне колекције у десном субдијафрагмалном простору, која је успешно третирана перкутаном дренажом. На послетку болесница је третирана хемиотерапијом, на коју је показала комплетан одговор потврђен контролним прегледима компјутеризованом томографијом.

Закључак Због својих неспецифичних клиничких, лабораторијских и радиолошких карактеристика примарни лимфом јетре може веома лако бити погрешно дијагностикован као неки чешћи примарни тумор јетре, те због тога треба бити разматран у диференцијалној дијагнози промена у јетри.

Кључне речи: хемиотерапија; дренажа; јетра; операбилан; тумор

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Acute type A aortic dissection – a case beyond the guidelines

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SUMMARY

Introduction There are not many cases among acute type-A aortic dissection survivors who get to be called "incredible." Here we present such a case followed-up for more than five years.

Case outline A 48-year-old male with acute type A aortic dissection, complicated with cardiac tamponade and severe aortic valve regurgitation, was submitted to emergent surgical treatment. Distal reconstruction was performed by complete aortic arch replacement with "elephant trunk" extension and separate arch branch bypasses, while the proximal reconstruction was done with Bentall procedure. Total of 11 anastomoses was necessary to complete this procedure. Straight profound hypothermic (18° C) circulatory arrest, with a saturation of the venous blood from the jugular bulb of 97%, lasted 133 minutes. The patient was discharged stable without any neuro-cognitive deficit. Two years later, he was admitted with late prosthetic valve endocarditis and subvalvular abscess. Good response on treatment with efficient combined antibiotics and stabile hemodynamic allowed us to avoid barely feasible re-do surgery. Subvalvular myocardial abscesses evolved into periprosthetic pseudoaneurysms without infectious, thrombo-embolic, or hemodynamic deterioration. The patient is still alive and stable, more than four years after this event.

Conclusion Fortunate outcome of these life-threatening conditions is a reason to reconsider our understanding of cerebral function and metabolism during the profound hypothermic circulatory arrest, and it emphasizes the importance of measuring individual patient response against disease treatment guidelines, as we did, treating the late, complicated prosthetic valve endocarditis with medicaments, instead of high-risk surgery.

Keywords: aortic dissection; circulatory arrest; brain protection; prosthetic valve endocarditis

INTRODUCTION

Experienced surgeons would agree that each case of acute type A aortic dissection (A-AAD) is peculiar. This emergent entity is well known as a dreaded disease with many faces, classified among the five most common misdiagnoses. [1] Surgical treatment strategies are permanently evolving to ensure appropriate reconstruction, with the lowest possible brain and visceral organ damage. Accordingly, mortality and morbidity rates, as well as long-term results, significantly improved [2, 3, 4]. Yet, there are not so many cases among survivors deserving the adjective "incredible." Here we present such a case of the A-AAD, followed-up for more than five years. The purpose of this report is not to recommend our treatment strategy, but to recall and expose all kind of concerns we had, celebrating the unexpectedly fortunate outcome of this "drama in two acts."

CASE OUTLINE

The first act: acute type A aortic dissection

A 48 years old male (195 cm, 92 kg), with a history of uncontrolled hypertension, was admitted with ongoing chest pain, hypotension (80/40 mmHg, sinus tachycardia 110/min), spontaneously breathing, and somnolent, with A-AAD, verified by multislice detector computed tomography (MDCT), complicated with cardiac tamponade and moderate-to-severe aortic valve regurgitation. Except for hypertension, no other known risk factors for A-AAD were present. Pain-to-table time was six hours, while diagnosis-to-table time was 45 minutes.

Median sternotomy and left common femoral artery access were done simultaneously, to relive severe tamponade and ensure fast arterial line placement. Following the massive coagulum removal from the pericardial sac, the two-stage venous cannula and left atrial vent was placed. Retrograde extracorporeal circulation was initiated, and the patient was cooled down to 18° C (straight profound hypothermia), under the alpha-stat protocol, without aortic clamping and cardioplegia. Bilateral ice-pads were placed over the carotid arteries and no additional pharmacological measures for brain protection were used. Cerebral blood oxygenation was monitored by near-infrared spectroscopy and saturation of the venous blood from the jugular bulb before the arrest was 97%. Aortotomy revealed multiple irregular tears in the ascending aorta and the arch, destroying orifices and proximal

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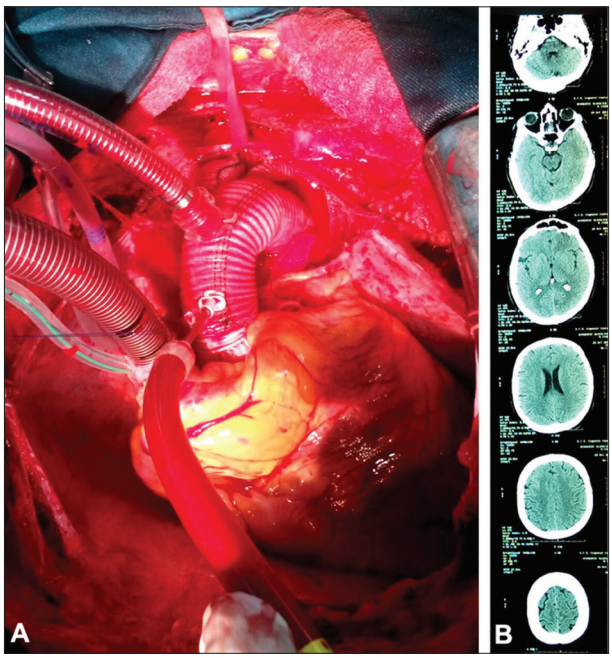

Figure 1. A – Completed procedure (11 anastomoses): proximal bentall + complete aortic arch and distal reconstruction (separate arch branches bypasses, distal "elephant trunk" descending aortic extension); B – postoperative brain multidetector computed tomography: absence of neurological and/or cognitive deficits; normal multidetector computed tomography

parts of all brachiocephalic arch branches. The left subclavian artery and proximal descending aorta were nearly occluded by the false lumen. At this point, there was no chance to change our initial straight profound hypothermic circulatory arrest and "the-arch-first" strategy, so that we had to go on with the "hostile" anastomoses at the distal aorta and arch branches. The distal aortic anastomosis was performed with inverted (26 mm) tubular Dacron graft, leaving an elephant-trunk extension of 7 cm in descending aorta. Arch reconstruction necessitated resection of proximal 3–4 cm of all three branches (all far above the left brachiocephalic vein) and interposition of small (10 and 8 mm) tubular Dacron grafts. Anterograde reperfusion was started by direct arterial cannulation of the distal aortic graft. (Figure 1A) Total straight profound hypothermic circulatory arrest (PHCA) time, without any cerebral perfusion, lasted for 133 minutes! Proximally, two of three commissures were detached so that Bentall procedure, using Valsalva Dacron composite graft (23 mm valve, 24 mm graft), was necessary. Overall, a total of 11 anastomoses were necessary to complete this procedure. Hemostasis was perfect and supported by fibrin sealant on each suture line. The patient was easily weaned from cardio-pulmonary bypass, without any hemodynamic support.

Extremely difficult pathoanatomy of the arch and extremely long PHCA time without any brain perfusion did not leave us any objective reason to be optimistic. Yet, on the fifth postoperative day, the patient woke up with no neurological or cognitive deficits. Brain MDCT was completely normal. (Figure 1B) Two weeks following the operation, significant pericardial and left pleural effusions were drained under fluoroscopic control, with no residual effusions on discharge. Control echocardiography (ECHO) revealed normal structural and functional parameters of the aortic valve and the left ventricle. He was discharged with beta-blocker (Presolol® 2 × 25 mg), ACE-inhibitor (Zorkaptil® 2 × 6.25 mg), Aspirin® (1 × 100 mg), anticoagulant (Farin® with target INR 2–3), Ibuprofen (Brufen® 3 × 600 mg), Colchicine® (2 × 0.5 mg), combined diuretics every other day: Furosemide (Lasix® 1 × 40 mg) plus Spironolactone (Aldactone® 1 × 25 mg), Cefixime (Pancef® 1 × 400 mg, one week) and Lansoprazole (Sabax 2 × 30 mg). Except for occasional hypertensive episodes, patient's condition and laboratory findings were excellent during the next two years follow-up. He went back to his usual physical and intellectual activities and actively took part in the "Aortic Disease Awareness Day" (ADAD) [5].

The second act: prosthetic valve endocarditis with subvalvular extension

Almost four years ago, after respiratory infection with prolonged fever (up to 40.2°C), fatigue, headache, and myoarthralgia, he was examined at the Emergency room and discharged with Erythromycin® (4 × 500 mg). Transthoracic ECHO did not reveal any prosthetic valvular pathology. A week later, he was admitted to the Clinic for infectious diseases, with the same symptoms and positive bio-humoral syndrome (Le 18.7, SE 74, Fib 6.3, CRP 177.6). The ECHO on admission revealed the presence of prosthetic valve endocarditis (PVE) with semicircular subvalvular abscess, affecting interatrial and interventricular septum. Repeated blood cultures were positive for coagulase-negative staphylococci (CoNS) and therapy was conducted combining different efficient antibiotics (vancomycin, levofloxacin, ciprofloxacin, linezolid, teicoplanin, amikacin). Subsequent ECHO and MDCT examinations revealed an emptied abscess cavity in a form of the periprosthetic pseudoaneurysm (with a maximal diameter of 20 mm) and normal prosthetic valve function (Figure 2A). Both the patient's good response to the treatment and our reluctance for a re-do surgery resulted in consent for "watchful-waiting" strategy and surgery just in case of any deterioration. After two months of therapy, he was discharged in a good clinical and hemodynamic condition with much better laboratory parameters (Le 6.7, SE 62, Fib 5.6, CRP 10.4).

During the next three years, there was no relapse of the PVE. Control ECHO and MDCT scans were scheduled every six months and revealed persistent, stationery periprosthetic pseudoaneurysm without any signs of thrombosis. The valvular function was normal as well as other functional parameters of the left ventricle (Figure 2B). Again, the patient is physically and intellectually active, still actively supporting the ADAD on September 19 each year.

Written consent for the publication of this case report and any accompanying images was obtained from the patient.

DISCUSSION

Acute aortic dissection, critical preoperative condition, extremely difficult pathological anatomy affecting ascending

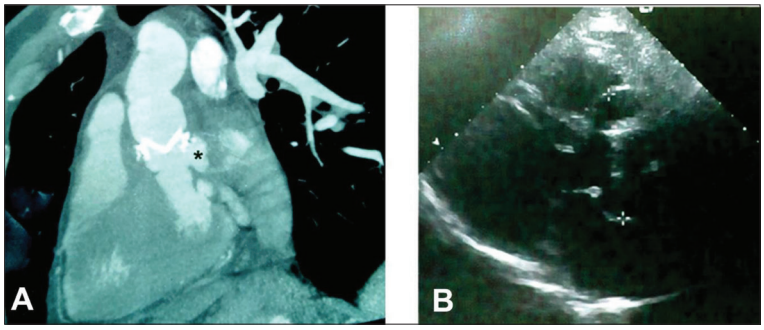

Figure 2. A – postoperative chest multidetector computed tomography: an emptied subvalvular abscess (black asterisk); B – postoperative echocardiography: periprosthetic pseudoaneurysm (white asterisks)

aorta, arch and branches, complex surgical reconstruction (i.e. 11 anastomoses), straight PHCA far above tolerable time limits (i.e. 133 minutes), postoperative pericardial and pleural effusion – all these things ("the first act") our patient survived without any serious consequences. Moreover, two years later ("the second act"), he also survived another life-threatening condition – PVE with perivalvular extension treated only with medicamentous therapy, with stabile course three years after. Both separately and especially in combination, these two "acts" indeed deserve the adjective "incredible." So far, there are no literature reports of similar cases.

As for "the first act," we want to emphasize the importance of time in the management of patients with suspected A-AAD [6] with or without malperfusion syndrome [7]. Accordingly, with a pain-to-table time of six hours and diagnosis-to-table time of only 45 minutes, we have managed to avoid serious malperfusion and multiorgan system failure. As for the operative strategy, it is well-known that cardiopulmonary bypass (CPB) induced PHCAs in humans, lasting > 40 minutes significantly increase the incidence of neurological deficits, while those of > 60 minutes significantly increase mortality caused by cerebral damage. [8, 9, 10] Swensson et al. [11] have reported 20.8% mortality and 14.6% stroke rates in 48 patients with PHCA 60–120 minutes. We could not find any literature report of straight PHCA > 120 minutes in humans. Yet, there are some reports of complete recovery after 6–8 hours of cardiac arrest from deep accidental hypothermia, but such cases could not be compared with CPB induced PHCAs [12, 13]. In addition, there were experimental studies, which did not find any cerebral pathologic changes or behavioral disorders after 90 and 120 minutes of PHCA in dogs, except hippocampal apoptosis following 120 minutes of PHCA at 15°C. Such a good ischemic tolerance may be attributed to non-thoracotomic CPB and the absence of hemodilution [14] Repeated CT scans did not reveal any brain damage in our patient. Though we did not use any tools to precisely measure his neurocognitive function, neither we nor his family members could proclaim it was impaired in any aspect. [15] Subsequent critical appraisal revealed that anterograde cerebral perfusion could be established earlier, by reconstructing the brachiocephalic trunk as the first step. We missed this opportunity being distracted by an impressive arch and branches pathology.

As for the "the second act," class I, level of evidence C recommendation for surgery in PVE claims: "Surgery is indicated for patients with PVE who present with complications, for example, abscess formation" [16]. Two years after the initial surgery, our patient developed late CoNS PVE with paravalvular extension. During this hospital stay, we were consulted daily to eventually decide for the re-do surgery. Our "out-of-the-box" watchful waiting attitude did not have any support in contemporary recommendations or some other literature reports [16, 17]. Good response on combined antibiotic therapy, stabile general and hemodynamic condition, on the one hand, and complex underlying pathology requiring very high-risk surgery, on the other, were the principal reasons for such approach. Not only the standard hazards of re-do surgery raised our concerns, but also some specific circumstances, such as the presence of coronary artery buttons (sealed with fibrin glue), uncertain geometry and histology of the periprosthetic pseudoaneurysm and adjacent myocardium. Guideline based surgery recommends complete reconstruction, including exclusion/excision of the pseudoaneurysm, prosthetic aortic valve and ascending aortic graft replacement with re-implantation of coronary artery buttons (or Cabrol modification) [16]. In the presence of scar tissue enhanced with fibrin sealants, such demanding procedure would be hardly feasible. A few years later, the report by Saitto and Russo [18], was the only one to compare with, to justify our concerns and decision made. Unlike their one-year follow-up experience, our patient is still alive with satisfactory clinical condition and hemodynamics, more than three years after the PVE (October 13, 2016) and more than five years after the initial operation (October 13, 2014).

A single case could never be a reason to build up an attitude or recommendations based on it. This particular case is definitively a reason to reconsider our understanding of cerebral function and metabolism during the PHCA conditions. A fortunate outcome with no neurological and/or cognitive deficits after 133 minutes of PHCA deserves it. Besides, this case emphasizes the importance of measuring individual patient response against disease treatment guidelines, as we did, deciding to treat the late, complicated CoNS PVE with medicaments, instead of high-risk surgery, fulfilling the Hippocratic injunction: *Primum non-nocere* (i.e., Above all, do no harm) [19].

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Акутна аортна дисекција тип А – случај изван препорука

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САЖЕТАК

Увод Не постоји много болесника који су преживели акутну дисекцију аорте тип А, а чији исход би оправдао епитет „невероватног случаја".

Циљ нам је да прикажемо један такав случај, који смо пратили више од пет година после операције.

Приказ болесника Мушкарац стар 48 година, са акутном дисекцијом аорте тип А, компликованом тампонадом и тешком аортном регургитацијом хоспитализован је ради неодложне кардиохируршке интервенције. Дистална реконструкција је подразумевала комплетну замену лука аорте са екстензијом *elephant trunk* уз одвојене бајпас реконструкције за све три гране лука, док је проксимална реконструкција захтевала извођење Бенталове операције. За комплетирање ове процедуре било је неопходно укупно 11 анастомоза. Директан дубоки хипотермни (18°C) циркулаторни застој, са сатурацијом венске крви из југуларног булбуса од 97%, трајао је 133 минута. Болесник је отпуштен у стабилном стању без неуро-когнитивних дефицита. Две године касније хоспитализован је под сликом ендокардитиса вештачке валвуле са субвалвуларним апсцесима. Добар одговор на терапију комбинованим ефикасним антибиотицима уз стабилну хемодинамику омогућио нам је да избегнемо реоперацију високог ризика. Субвалвуларни апсцеси су еволуирали у перипростетичну псеудоанеуризму без знакова инфекције, тромбо-емболијских и хемодинамских погоршања. Болесник је жив, у стабилном стању, више од четири године после ове компликације.

Закључак Исход лечења овог болесника је довољан разлог да преиспитамо наше разумевање церебралне функције и метаболизма у условима током дубоког хипотермног циркулаторног застоја, као и да нагласимо важност индивидуалне процене ризика сваког болесника, чак и кад се он значајно разликује од актуелних препорука, као у овом случају, кад је компликовани ендокардитис вештачке валвуле лечен антибиотицима, уместо хируршки.

Кључне речи: аортна дисекција; циркулаторни застој; протекција мозга; ендокардитис вештачке валвуле

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Ligamentum flavum hypertrophy in a patient with Pott's disease

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SUMMARY

Introduction The spine is involved in less than 1% of all tuberculosis (TB) cases, and it is a very dangerous type of skeletal TB as it can be associated with neurologic deficit and even paraplegia due to compression of adjacent neural structures and significant spinal deformity. The spine TB is one of the most common causes for an angular kyphotic deformity of spine. Patients with kyphosis angle ≥ 60° at dorsolumbar spine are at great risk to develop late onset neurological deficit and paraplegia due to chronic compression and stretching of the spinal cord over bonny ridges. In a small number of cases, other conditions may lead to neurological deficit in patients with long standing angular kyphosis which also alters the treatment strategy that otherwise involves prolonged and mutilant surgery.

Case outline We present a case of a 61-year-old male patient with concomitant 90° dorsolumbar spine kyphosis due to spinal TB and ligamentum flavum hypertrophy which led to spinal canal stenosis with myelopathy, and consequent paraplegia. The patient underwent dorsal decompression with hypertrophic yellow ligament removal after which he recovered to the level of walking.

Conclusion Many authors propose guidelines for treatment of spinal TB, taking into account the stage of the disease, the age of the patient, the angle of kyphosis, and other factors. We find that personalized medical approach is the best approach for each patient.

Keywords: kyphotic deformity; late onset paraplegia; TB spine; spinal canals stenosis; flavum hypertrophy

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases affecting humans and it has been found in the ancient mummies of Peru and Egypt. The disease is caused by the bacillus *Mycobacterium tuberculosis*, and occasionally by *Mycobacterium africanum* or *Mycobacterium bovis*. The spinal column is involved in less than 1% of all TB cases. First case was described in 1779. by Percival Pott, and since then the disease is frequently called Pott's disease. Main reasons for late onset paraplegia occurrence in patients who had Pott's disease are long standing angular kyphosis and chronic compression and stretching of the spinal cord over bonny ridges [1]. However, in a small number of cases, other conditions may simultaneously be present besides spinal deformity and result in spinal canal stenosis and neurological deficit.

We present a patient with concomitant hyperkyphosis due to spinal TB and ligamentum flavum hypertrophy.

Written consent was obtained from the patient to publish all shown material. This study was done in accordance of the institutional standards on ethics.

CASE REPORT

We present a 61-year-old male Caucasian, presented to our clinic with long-term history of chronic back and leg pain. When he was three years old, he was treated for spinal TB in an orthopedic clinic in another country, but no medical documentation is available. It was decided not to operate at that time, and patient was recommended to use braces. Patient states that he was inconsistent in going to checkups. As a consequence of irregular treatment pronounced gibbus in thoracolumbar spinal region developed. However, he had no symptoms or difficulties of any kind. He was physically active, played football since he was eight years old, graduated from high school and was employed. At the age of 57, pain in his back appeared. Pain was worsening at night, and, with time his foot started dropping. He was admitted to the Spinal orthopedic center in Belgrade, where first spine X-ray was performed and gibbus deformity with thoracolumbar kyphosis resulting in 90° angulation was observed (Figure 1). After that, spine a magnetic resonance imaging (MRI) was performed, which showed a kyphotic deformity, but with normal signs of myelon, according to radiologist's description (patient lost this MRI images), so the patient was treated conservatively. He was pain free for three years, but in 2009 the pain worsened, predominantly in the right leg, however, he did not receive any treatment. In 2013, the patient started experiencing problems while walking and within a few months he developed paraplegia. Spine MRI was performed and clearly demonstrated myelopathy finding at the level

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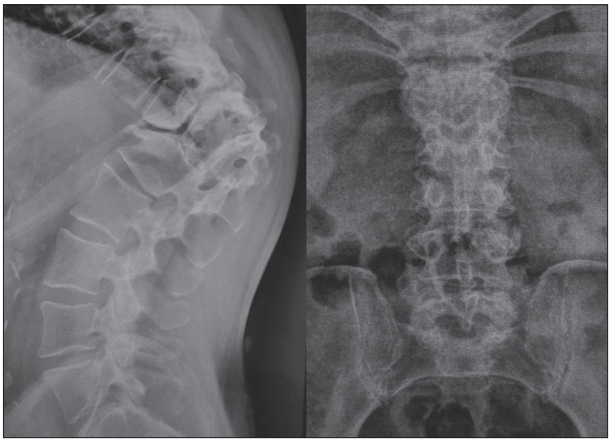

Figure 1. X-ray of the spine showing severe kyphosis at the thoracolumbar junction

of Th11–Th12 with hypertrophied yellow ligament at the same level (Figure 2). He was offered surgical treatment, but as procedure was supposed to include anterior decompression and spinal fusion, patient refused treatment at that time. After several consultations with spinal orthopedic surgeons and neurosurgeons from other institutions, the patient was operated by posterior decompression via partial laminectomy and excision of hypertrophied ligamentum flavum at the level of Th11–Th12 consistent with the level of myelopathy, without other procedures. Following the surgery, the patient gained ability of cane aided gait. Early control spine MRI showed signs of good spinal canal decompression at the level of Th11–Th12, with regression of myelopathy signs. A few months after the operation, the patient started experiencing lumbar pain and leg numbness with pain more pronounced in right leg, which is why he was further, evaluated at our department. Patient reported pain on visual analog scale 5/10, objectively he had bilaterally negative Lazarević's sign (Lasègue test), normal muscle tonus, with symmetrically reduced myotonic reflexes, and normal plantar flexion response, normal muscle strength, no objective loss of sensation and antalgic cane-aided gait. Kyphosis in upright posture was evident. A control computed tomography scan was performed and showed good decompression, without signs of spine instability or progression of kyphosis (Figure 3). Since no further neurological deficit has been developed and based on neuroradiology findings, it was concluded that no further surgical treatment was necessary, caudal epidural blockage was performed in local infiltrative anesthesia and patient was discharged in good condition with significantly reduced pain and better walking (Figure 4).

DISCUSSION

There are two basic types of spinal TB. The first is the classic form or spondylodiscitis. The second one is atypical form, which is spondylitis without involvement of intervertebral disc. The basic lesion in Pott's disease is a combination of arthritis and osteomyelitis, usually affecting more than one vertebra, and most commonly involving anterior aspect of the vertebral body. Spinal TB can include progressive bone destruction leading to kyphosis and vertebral collapse, formation of cold abscess, spinal

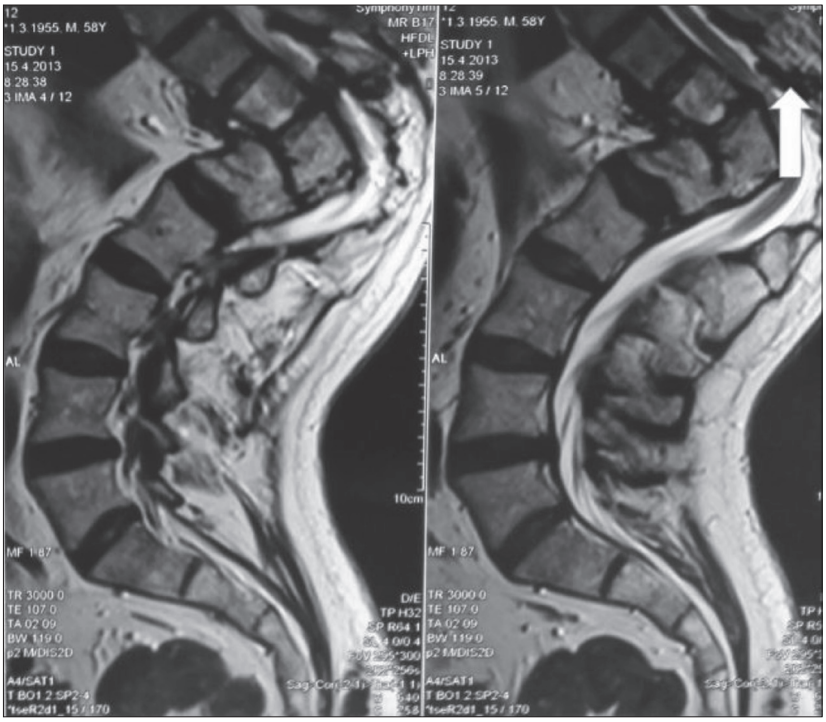

Figure 2. T2 weighted magnetic resonance imaging of the thoracolumbar spine showed pronounced kyphosis with signs of spinal canal stenosis most pronounced at the level of Th11–Th12 with the consequent myelopathy finding, due to hypertrophied yellow ligament at the same level (arrow)

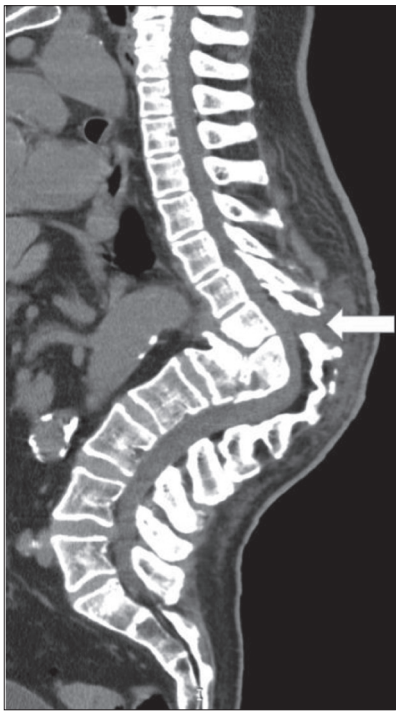

Figure 3. Control computed tomography scan of the spine showing good decompression at the level of Th11–Th12 (arrow), without signs of instability or progression of kyphosis

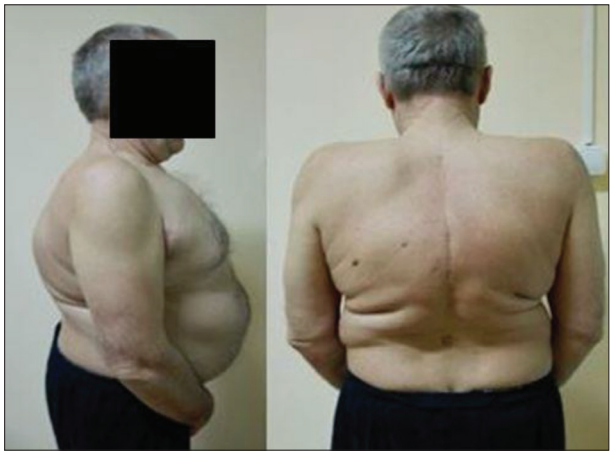

Figure 4. The patient has kyphosis in upright posture (since childhood); after the operation, patient can stand alone and walk with a cane; pronounced gibbus is seen in the thoracolumbar region; on the right picture a scar from surgery in the thoracolumbar region is visible

canal narrowing by granulation tissue, abscesses, or direct dural invasion resulting in spinal cord compression and neurologic deficits [2].

Pott's disease is one of the most common cause for an angular kyphotic deformity of spine, particularly in developing countries. There is an average increase in spine kyphosis of 15° in all patients treated conservatively, and a deformity greater than 60° can develop in about 3% of patients [3]. Children are more prone to develop greater deformity, probably due to the cartilaginous nature of their bones. The development of kyphosis occurs in two stages of disease: (phase I) during active disease and infection, (phase II) after healing of the lesion. The deformity developed in the phase II with neurologic deficit has worse prognosis than complications that occur during the phase I [4]. In our case, kyphosis developed in the early stage of the disease, but paraplegia occurred at a late stage of the disease, due to hypertrophy of the yellow ligament causing stenosis of the spinal canal and the consequent compression of the spinal cord.

The progression of kyphosis depends on number of vertebrae involved during phase I of the disease, initial vertebral body loss and segment of spine affected. There is some evidence that if patients developed 60° or more kyphosis at dorsolumbar spine they were likely to develop late onset paraplegia [5]. There are several risk factors, which may indicate severe progression and patients that are at great risk of deformity progression, such as: age under ten years and loss of one or one and a half vertebral bodies (I), a pre-treatment kyphosis angle of greater than 30°, especially in children (II), thoracolumbar junction lesions (III), radiological signs of "spine at risk" (IV) [6]. These signs of "spine at risk" are: separation of the facet joints (a), retropulsion of vertebras (b), lateral translation (c), and toppling (d). These signs are manifestation of spinal instability due to dislocation of the facet joints. Each of these signs is given a score of one. Total score of three or four can predict an increase in the kyphosis by more than 30° and a final deformity of more than 60° [7]. Since our patient was inconsistent with medical checkups, the disease treatment was inadequate. According to the mentioned risk factors, our patient was at great risk for severe deformity development, which happened, and final manifestation was paraplegia. Analyzing radiological findings of our patient, it is most likely that he had all four signs of "spine at risk", finally resulting in 90° kyphosis. Also, patients with pronounced kyphosis are prone to develop severe spinal canal stenosis, probably due to compensatory ligament and bone hypertrophy in the spine that is instable. Persistent deformity affects the biomechanics of all spine segments [8]. This is what happened to our patient, and which in the end turned out to be the biggest problem in our patient, since he developed myelopathy due to spinal canal stenosis.

The indications for surgery in Pott's disease are patients with neurologic deficit. Although management of patients with active disease is well defined, there is a lack of literature on the management of spinal kyphotic deformity caused by TB [9]. Combined posterior and anterior osteotomy, correction of deformity, and instrumented fusion are shown to arrest progression of kyphosis and improve neurologic symptoms. Different techniques have been used to correct the kyphotic deformity. A single-stage posterior Smith-Peterson osteotomy, pedicle subtraction osteotomy, vertebral column resection through a single-stage anterior–posterior approach (anterior decompression followed by posterior instrumentation), direct internal kyphectomy and other new approaches are mostly used techniques, but these procedures are associated with significant blood loss, major complications and high morbidity [9, 10]. In the cases of patients with severe and long-standing kyphosis, who were treated for spinal TB 15 or more years ago, and with new presentation of paraplegia or upper motor neuron spinal cord injury, anterior decompression and fusion is advocated [10, 11, 12]. Our patient was a candidate for this procedure, but after being presented with all possible complications, length of surgery and hospital stay, he refused the treatment. He was than admitted to our neurosurgical department, and after additional analysis of the spine MRI, and after consultations with spinal orthopedic surgeons and neurosurgeons from other institutions, patient was offered posterior decompression with only partial laminectomy and with removal of hypertrophied yellow ligament, without kyphus correction or fusion. Our presumption that the problem was mainly due to hypertrophied yellow ligament was set on the basis that the kyphotic deformity did not change over several years, and that myelopathy sign was present at the same level where yellow ligament was thickened. Also, since our patient did not have restriction of pulmonary functions, we decided that best approach was partial laminectomy with flavectomy, which proved to be a successful approach, since the patient achieved neurological status improvement and good recovery.

Hyperkyphotic deformity remains the main reason for late onset paraplegia after spinal TB. However, other causes such as ligamentum flavum hypertrophy can simultaneously occur and contribute to progressive spinal canal stenosis and compression of the cord, causing a neurological

deficit. Although, many authors propose guidelines for treatment of spinal TB taking account phase of the disease, patient's age, kyphosis angle and other factors, we advocate personalized approach to every patient. In addition, many complications, such as kyphosis can be avoided by early diagnosis of spine TB and by proper treatment. So, patient with cured TB must be controlled with regular checkups in order to prevent late complications.

Conflict of interest: None declared.

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Хипертрофија жутог лигамента код болесника са Потовом болешћу

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САЖЕТАК

Увод Кичма је укључена у свега 1% случајева туберкулозе и овај скелетни облик туберкулозе може бити удружен са неуролошким дефицитом или чак са параплегијом услед компресије на нервне структуре и значаног деформитета кичме. Спинална туберкулоза је један од најчешћих узрока угаоног кифотичног деформитета кичменог стуба. Болесници са углом кифозе од преко 60° у грудно-слабинској регији су у великом ризику да у каснијем току болести развију неуролошки дефицит и параплегију услед дуготрајне компресије и истезања кичмене мождине преко коштаних структура. У малом броју случајева постоје други узроци неуролошког дефицита код болесника са дуготрајном угаоном кифозом, што мења стратегију лечења, која иначе подразумева дуготрајне и мутилантне операције.

Приказ болесника Приказујемо случај мушкарца старости 61 годину са израженим кифотичним деформитетом са углом од 90° у тораколумбалној регији насталим услед спиналне туберкулозе прележане у раном детињству и хипертрофијом жутог лигамента, који је довео до стенозе спиналног канала удружене са мијелопатијом и последичном параплегијом. Болеснику је урађена дорзална декомпресија у смислу уклањања хипетрофичног жутог лигемента, после чега се болесник опоравио до нивоа самосталног хода.

Закључак Многи аутори предлажу водиче за лечење спиналне туберкулозе узимајући у обзир фазу болести, старосну доб болесника, угао кифозе, као и друге факторе. Ми сматрамо да је персонализовани медицински приступ најбољи приступ сваком болеснику.

Кључне речи: кифотични деформитет; параплегија касног почетка; туберкулоза кичме; стеноза спиналног канала; хипертрофија жутог лигамента

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Renal cell carcinoma of a horseshoe kidney

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SUMMARY

Introduction Horseshoe kidney is the most common developmental disorder of the urinary system, which involves an anomaly of kidney fusion, and occurs in 3% of the population. Kidneys are most often connected at the lower poles by fibrous or parenchymal isthmus. Renal cell carcinoma (RCC) is the most common tumor of the horseshoe kidney. Treatment involves surgical treatment that includes heminephrectomy or partial nephrectomy with different approaches. We report a case of RCC of a horseshoe kidney, located on the lower pole and the isthmus.

Case outline A 68-year-old patient reported to the urologist due to intermittent painless macroscopic hematuria. Computed tomography urogram revealed the presence of a tumor on the right kidney, measuring 85 × 90 × 60 mm, with radiological characteristics of RCC, which covered the entire lower pole of the kidney towards the isthmus. Angiography finding indicated a thickened isthmus with pronounced malformation of vascular structures. The right heminephrectomy was performed with resection of the isthmus from 15 mm to the healthy tissue. The isthmus was sutured in two layers with a catgut suture. Subsequently, hilar, paracaval, and interaortocaval lymphadenectomies were performed. The pathohistological finding indicated a tumor of renal cell origin, while the resection line was free of the tumor tissue, as were the lymph nodes.

Conclusion RCC is the most common neoplasm of the horseshoe kidney. Treatment is surgical and involves open or laparoscopic heminephrectomy or partial nephrectomy with a transperitoneal or extraperitoneal approach.

Keywords: horseshoe kidney; renal cell tumor; nephrectomy

INTRODUCTION

Horseshoe kidney is the most common developmental disorder of the urinary system, which involves an anomaly of kidney fusion, and occurs in 3% of the population. This anomaly occurs in the fetal period, between the fourth and sixth weeks of life, and occurs in 1–4 people per 1000 births [1]. Horseshoe kidney occurs more often in men than in the female population in the ratio of 2:1. Developmental disorders of other parts of the urogenital tract as well as other organ systems may be associated with the appearance of the horseshoe kidney. Kidneys are most often connected at the lower poles by the fibrous or the parenchymal isthmus, which can have its own blood vessels [2].

Renal cell carcinoma (RCC) is the most common tumor of the horseshoe kidney, but the risk of developing of this disease is similar to the risk in kidneys without developmental disorders. The highest incidence of RCC is in the seventh and eighth decades of life, while the most common risk factors for RCC are tobacco exposure, obesity, and hypertension. The risk of developing Wilms' tumor and tumors of transitional epithelium increases two to six times in horseshoe kidneys [3]. The diagnosis of the horseshoe kidney is most often made accidentally due to the examination of other diseases and conditions such as arterial hypertension. Symptoms that may indicate the presence of a malignant process are hematuria and nonspecific abdominal pain. Diagnostic procedures include ultrasonographic examination, cystoscopy, angiography, computed tomography (CT) with urography and nuclear magnetic resonance of the abdomen and the lesser pelvis [2, 3].

Treatment involves surgical treatment that includes heminephrectomy or partial nephrectomy with different approaches. These surgical procedures can be complicated by the presence of pathological vascularization and the impossibility of resection of the isthmus. We report a case of RCC of a horseshoe kidney, located on the lower pole and the isthmus.

CASE REPORT

A 68-year-old patient reported to the urologist due to intermittent painless macroscopic hematuria that lasted two months. Complete blood and biochemical analyses were within the reference values. The urine culture was sterile. Arterial hypertension was common comorbidity. Ultrasonographic examination revealed a tumor mass of the right kidney measuring over 80 mm, which extended interpolarly towards the lower pole of the kidney. The lower pole of the kidney was connected to the left kidney by an isthmus. An ultrasound examination indicated a dilatation of the pyelocalyx system of the right kidney, while the pyelocalyx system of the left kidney

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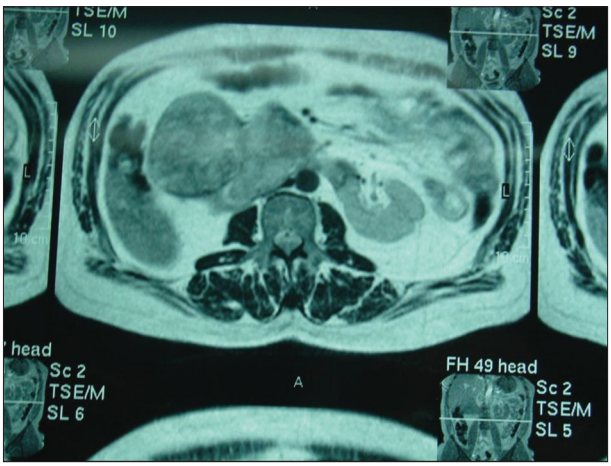

Figure 1. Computed tomography urography showed the presence of a tumor on the right kidney measuring 85 × 90 × 60 mm, with radiological characteristics of renal cell carcinoma, which covered the entire lower pole of the kidney towards the isthmus

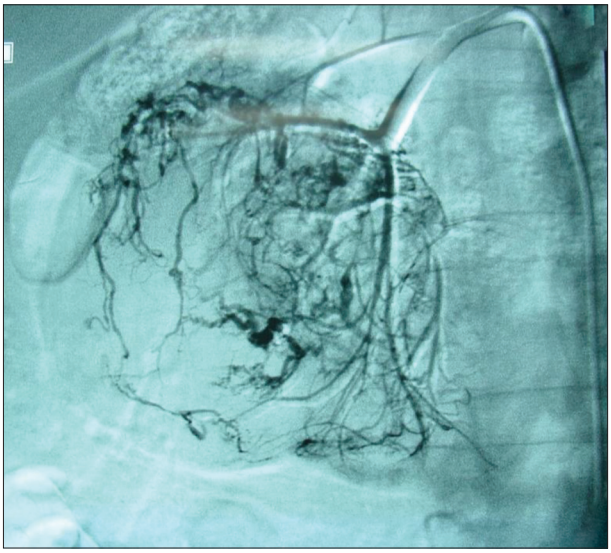

Figure 2. Angiography finding indicated a thickened isthmus with pronounced malformation of vascular structures

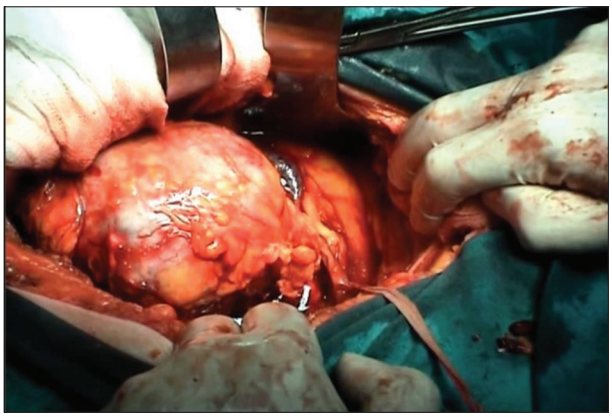

Figure 3. Right heminephrectomy by extraperitoneal approach, with an enlarged lumbotomy incision and resection of rib XI

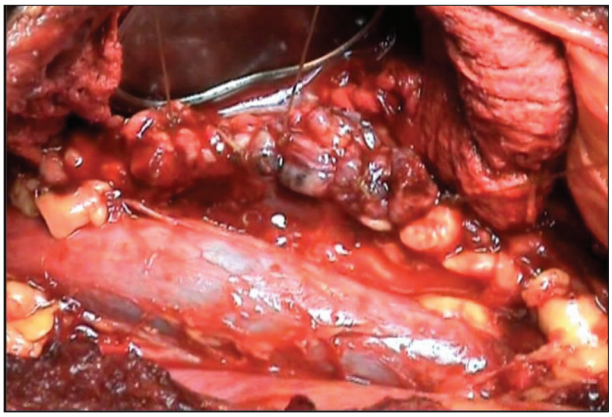

Figure 4. The isthmus was sutured in two layers with a catgut suture; hilar, paracaval, and interaortocaval lymphadenectomies were performed

was normal, without calculosis. Next, CT urography was performed, which showed the presence of a tumor on the right kidney, measuring 85 × 90 × 60 mm, with radiological characteristics of RCC, which covered the entire lower pole of the kidney towards the isthmus (Figure 1). The pyelocalyx system of the right kidney and the initial part of the right ureter were dilated. The left pyelon and the left ureter along the entire length of the course were normal. The angiography finding indicated thickened isthmus with pronounced malformation of vascular structures (Figure 2). It was decided to perform a right heminephrectomy by extraperitoneal approach, with an enlarged lumbotomy incision and resection of rib XI (Figure 3). The right kidney with the tumor mass, the right ureter, the inferior vena cava, and the entire isthmus were dissected and the left ureter was identified. Next, a right heminephrectomy was performed with resection of the isthmus from 15 mm to the healthy tissue. A sample of isthmus tissue from the resection line was sent for pathohistological analysis. The isthmus was sutured in two layers with a catgut suture (Figure 4). The hemostasis was correct. Subsequently, hilar, paracaval, and interaortocaval lymphadenectomy were performed. The postoperative course was uneventful, and the patient was discharged from the hospital on the 10th postoperative day. The pathohistological finding indicated a tumor of renal cell origin, while the resection line was free of the tumor tissue, as were the lymph nodes. After three years, a control CT finding of the abdomen, the pelvis, and the chest showed no local recurrence of the disease or the presence of secondary deposits.

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.

DISCUSSION

Horseshoe kidney is the most common anomaly of kidney fusion and it is more common in men than in women. Its frequency is estimated at about 0.25% of the total population. Genetic predisposition has not been proven, although this anomaly has been identified in twins and siblings within the same family. The horseshoe kidney consists of two different functional kidneys on each side of the medial

line of the body, which are connected to the lower poles by the isthmus. The isthmus contains blood vessels, functional tissue of the renal parenchyma, or connective tissue [4, 5].

Renal cell tumor is the most common neoplasm that occurs on the horseshoe kidney in about 50% of cases, while transitional cell carcinoma and Wilms' tumor are present in about 25% of cases. The etiological factors are not completely known, but the carcinogenesis of RCC cannot be related to the presence of a congenital defect [6]. Predisposing factors for the development of transitional cell carcinoma of the horseshoe kidney are obstruction, chronic infection and the presence of calculosis. The tumor can be localized in any part of the kidney, but is mostly located on the isthmus [1, 6]. In 1976, David Buntley presented 111 cases of tumors that developed in horseshoe kidneys, where RCC had the highest frequency [1]. Similar data have been published in contemporary literature.

The presence of this anomaly is in most cases asymptomatic and is detected incidentally in the process of diagnosing other processes and conditions. Symptoms are associated with hydronephrosis, infection, and calculus formation. Hematuria and nonspecific abdominal pain may indicate horseshoe kidney malignancy [7]. Abdominal aortic aneurysms and ovarian tumors must be considered as differential diagnoses. Diagnosis of horseshoe kidney tumors involves the same radiological methods used in the diagnosis of physiological development of kidney diseases, which include ultrasonographic examination of the abdomen and the lesser pelvis, CT urogram, angiography and nuclear magnetic resonance of the abdomen and the lesser pelvis [7, 8].

Heminephrectomy is indicated for the localization of tumors in the central part of the kidney, for tumors of larger dimensions, tumors in unfavorable position to the pyelocalyx system and vascular stem, in cases of a thicker and highly vascularized isthmus, as well as in elderly patients. Heminephrectomy usually involves a transperitoneal approach, while in certain cases the method can be reported with an extraperitoneal lumbotomy approach [4, 9].

If the tumor mass is localized at the poles, and its dimensions do not exceed 40 mm, the method of choice is sparing surgery, partial nephrectomy, which involves extraperitoneal and transperitoneal approach, or laparoscopic method using the same approaches [10]. When setting the indication for partial nephrectomy, three important characteristics of the horseshoe kidney must be taken into account: malformations of blood vessels, parenchymal or fibrous structure of the isthmus, and the anatomical position of the kidney itself [11]. In about 30% of cases of horseshoe kidney, one renal artery was observed, while a large number of vascular anomalies are found in about 70% of cases. The structure of the isthmus and the malformations of the blood vessels that supply it bear great influence on the choice of surgical method [12, 13]. In certain cases, preoperative superselective embolization of pathological blood vessels of the tumor can be performed, which enables a more efficient and safer surgical treatment [14].

In the presented case, the tumor measuring over 80 mm affected the entire lower pole and the isthmus, which was extremely thickened with a large number of blood vessels, so it was decided to perform heminephrectomy by the extraperitoneal approach, with an enlarged lumbotomy incision and resection of rib XI.

RCC is the most common neoplasm of the horseshoe kidney. The incidence of this tumor on the horseshoe kidney is not higher in relation to the population with normal fetal kidney formation. Diagnostic methods and prognostic factors are the same as for kidney tumors with undisturbed development. Treatment is surgical and involves open or laparoscopic heminephrectomy or partial nephrectomy with a transperitoneal or extraperitoneal approach. Significant characteristics of the horseshoe kidney that must be taken into account: malformations of blood vessels, parenchymal or fibrous structure of the isthmus, and the anatomical position of the kidney itself, which can determine the type of surgical treatment.

Conflict of interest: None declared.

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Карцином бубрежног паренхима на потковичастом бубрегу

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САЖЕТАК

Увод Потковичаст бубрег је најчешћи развојни поремећај мокраћног система који подразумева аномалију фузије бубрега и јавља се код 3% становништва. Бубрези су преко доњих полова повезани истмусом, који може бити изграђен од везивног или паренхимског ткива бубрега. Карцином бубрежног паренхима најчешћи је тумор на потковичастом бубрегу. Лечење је хируршко и подразумева хеминефректомију или парцијалну нефректомију са различитим приступима. Приказујемо случај карцинома бубрежног паренхима потковичастог бубрега, локализованог на доњем полу и истмусу.

Приказ болесника Шездесетосмогодишњи болесник јавио се урологу због повремене безболне макроскопске хематурије. КТ урографија је показала присуство тумора на десном бубрегу, димензија 85 × 90 × 60 *mm*, са радиолошким карактеристикама карцинома бубрежног паренхима, који је заузимао цео доњи пол бубрега према истмусу. Налаз ангиографије указивао је на задебљани истмус са израженом малформацијом васкуларних структура. Изведена је десна хеминефректомија са ресекцијом истмуса од 15 *mm* до у здраво ткиво. Истмус је ушивен у два слоја са катгутним шавом. После тога су урађене хиларна, паракавална и интераортокавална лимфаденектомија. Патохистолошки налаз указивао је на тумор порекла бубрежних ћелија, док је линија ресекције на истмусу била без туморског ткива, као и лимфни чворови.

Закључак Карцином бубрежног паренхима је најчешћи тумор потковичастог бубрега. Лечење је хируршко и укључује отворену или лапароскопску хеминефректомију или парцијалну нефректомију са трансперитонеалним или екстраперитонеалним приступом.

Кључне речи: потковичаст бубрег; карцином бубрежног паренхима; нефректомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Esophageal achalasia in a two-year-old boy

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SUMMARY

Introduction Esophageal achalasia is a neurodegenerative motility disorder, which is characterized by ineffective or absent esophageal peristalsis and the lack of hypertonic lower esophageal sphincter relaxation. Achalasia causes failure to thrive in children and can have serious respiratory complications. Achalasia is a very rare condition in pediatric population, and usually misdiagnosed as gastroesophageal reflux. The treatment of choice is Heller esophagocardiomyotomy.

The aim of this paper is to present a rare case of a two-year-old child with achalasia, diagnostic procedures, and successful operative treatment.

Case outline The patient´s problems started at the age of six months, with audible breathing and respiratory stridor. The child was admitted at a local hospital at seven months of age, dismissed with dietary advices, again admitted at the age of 19 months, and transferred to our institution. Upper gastrointestinal series and computed tomography revealed findings characteristic for achalasia, and on esophagoscopy exam there was no opening of lower esophageal sphincter and cardia on insufflation. Pneumatic dilation was performed with temporary improvement. Laparotomic Heller esophagocardiomyotomy with Dor partial fundoplication was successfully performed.

Conclusion Achalasia is a very rare condition in infants and young children. There is often a delay when establishing the correct diagnosis. Upper gastrointestinal series and endoscopic exam are most reliable methods to detect achalasia. Pharmacological treatment, intrasphincteric injection of botulinum toxin and pneumatic dilations are not efficient methods, especially in small children. The method of choice in the treatment of achalasia is Heller esophagocardiomyotomy with partial fundoplication.

Keywords: achalasia; children; surgery

INTRODUCTION

Esophageal achalasia is a neurodegenerative motility disorder of the esophagus, characterized by ineffective esophageal peristalsis and the absence of relaxation of hypertonic lower esophageal sphincter (LES), due to degenerative changes of inhibitory fibers and ganglion cells of esophageal Auerbach myenteric plexus [1, 2]. The clinical symptoms of achalasia were first described by Thomas Willis in 1674. The term achalasia was introduced by A. F. Hertz in 1914 and Hurst in 1927. The term cardiospasm has also been used for a long time. Some authors believe that achalasia could arise due to the disorder of extraesophageal innervation via the dorsal nuclei of the vagal nerve [2]. Cassella showed that in 68% of esophageal samples of patients with achalasia, the degeneration, or absence of ganglion cells were present [3].

The incidence of achalasia is 1:100,000 people, with symptoms mainly occurring in adolescents and adults between the ages of 30 and 60 [1, 4, 5]. Only 3–5% of patients are younger than 14 years, and the disease is extremely rare in infancy [5]. However, a case of premature infant weighing 1200 gr with achalasia is reported [5]. Some authors found the annual incidence of achalasia in children 0.11:100,000, mostly between the ages of seven and 12 [1]. Achalasia can be a primary congenital disorder, a consequence of some infectious diseases (Chagas disease, varicella and viral esophagitis), or autoimmune diseases (Guillain–Barre acute polyradiculoneuritis, Sjögren syndrome, eosinophilic esophagitis and scleroderma) [2]. Achalasia can be a part of Allgrove syndrome, together with alacrima and adrenal insufficiency (triple A) [4]. Some authors reported cases of transitory achalasia in infants with low birth weight or with Pierre Robin malformation [4]. Achalasia causes dysphagia, regurgitation, vomiting, aspiration of esophageal content with respiratory problems, anorexia, and failure to thrive. This disease can be complicated by obstructive bronchiolitis, lung abscess, pleural empyema, and pulmonary fibrosis.

The diagnosis of achalasia is established by esophageal manometry [6], esophagoscopy, upper gastrointestinal (GI) series and computed tomography (CT). The treatment can be conservative or surgical. Conservative treatment consists of lowering LES pressure pharmacologically (anticholinergic and antispasmodic medicaments) or by intrasphincteric injection of botulinum toxin. Nowadays, the conservative treatment can be applied only in very mild to moderate forms of achalasia, usually with unsatisfactory long-term effects [4]. Pneumatic dilation (PD) of the LES also can give only temporary

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positive results. The surgical treatment consists of Heller esophagocardiomyotomy (HE). The longitudinal splitting of smooth muscular fibers is made in the distal esophagus, cardia, and gastric fundus. An antireflux procedure should be performed at the same time, preventing postoperative gastroesophageal reflux (GER). Nowadays, HE is considered the treatment of choice for achalasia, especially in children.

CASE REPORT

The patient's problems started at the age of six months, when the intake of mushy and firmer foods began. Anamnestic data suggested that liquids and milk intake was pretty well tolerated. After food intake there was audible breathing, wheezing and respiratory stridor, without cyanosis. The child was admitted to a local hospital at seven months of age, lung radiographs, laboratory analyzes and otorhinolaryngological (ORL) examination were performed, and the patient was dismissed home with medical advice concerning food intake. At the age of 19 months the child was admitted again, and then transferred to our hospital. On admission, the child was malnourished, with body weight of 10.7 kg and body height of 83 cm. Lung auscultation revealed inspiratory stridor. Early psychomotoric development was regular. The patient's father was operated on because of hypertrophic pyloric stenosis. Pulmonary radiograph exam showed hilifugal bilateral salient interstitial drawing. Upper GI series revealed the markedly dilated esophagus, while it´s terminal part tapered conically and contrast, in a thin jet and in small amounts, passed into the stomach (Figure 1). Endoscopic exam revealed remained food in esophageal lumen, the dilated esophagus, and stenotic cardia not opening to insufflation. Esophageal narrowing at the level of the diaphragm was dilated by esophageal balloon N°10–12 to 2/3 of balloon width. For the first two weeks after dilation, the child's condition improved, but the recurrence occurred. The child was admitted again after 50 days, with the same complaints. The planned esophageal manometry at another institution could not be done because of the child's age. Chest CT scan recorded the dilation of the cervical and thoracic parts of the esophagus, with a maximum diameter of 17 mm in the anteroposterior (AP) direction, and 27 mm in the laterolateral (LL) direction. The esophageal wall was uniformly 3 mm thick, and hydroaeric level in the lower esophagus was prominent. When the patient was two years old, he was operated on. After medial laparotomy, the thoracic esophagus was mobilized, the vagal nerve identified and trapped. The longitudinal myotomy was performed, engaging 3 cm of the distal esophagus, cardia and 3 cm of the gastric fundus (Figure 2). The cardiomyotomy was difficult to perform due to the scar tissue, caused by previous PD. Esophageal and gastric mucosal prolapse occurred. The Dor anterior gastric fundoplication in the length of 2.5 cm was performed. Postoperative course was uneventful. Control upper GI series showed no contrast extralumination, esophageal dilation was still present, but contrast passed into the stomach faster and in a thicker jet (Figure 3). The patient was readmitted two months later. Food intake was significantly better and major complaints reduced. Contrast upper GI study showed uniformly expanded esophagus, and the distal part of the esophagus moderately narrowed, but barium passed into the stomach without delay. There were no signs of GER.

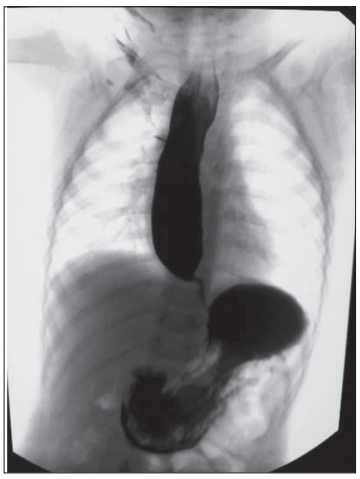

Figure 1. Preoperative radiography

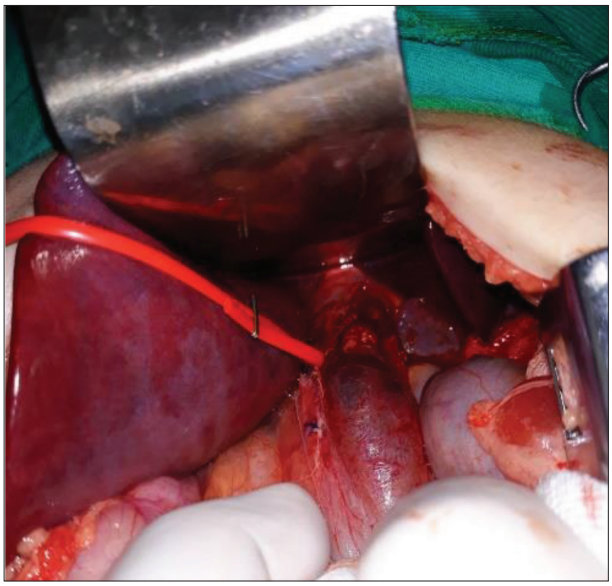

Figure 2. Operative myotomy

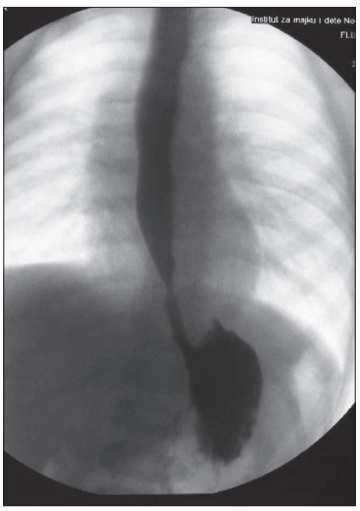

Figure 3. Postoperative radiography

Esophageal PD was performed and moderate stenosis at the cardia level completely dilated by the balloon 10–12 mm. After that, in six months follow-up the patient had no complaints or swallowing problems.

DISCUSSION

Esophageal achalasia in children is a rare disease and is often misdiagnosed as GER or, less commonly, as eating disorders, idiopathic failure to thrive, or bronchial asthma. Many studies have shown that achalasia in infants and young children manifests with difficulty feeding, nocturnal cough, wheezing, stridor, acute upper respiratory tract obstruction and recurrent respiratory infections, and in older children with symptoms of GER, sore throat, cough and hoarseness. Overall, the most presenting symptoms are regurgitation (83%), dysphagia (71%), poor growth (54%) and respiratory symptoms (41%) [7]. The diagnosis of achalasia is difficult to establish in children under five years of age, since its incidence is very low, and often there is a delay in making the diagnosis and applying the appropriate treatment [1]. Although 18% of pediatric patients have symptom onset during infancy, in only 6% of patients the diagnosis of achalasia is established during infancy [7]. In a meta-analysis made by Myers et al. [7], performed on 175 pediatric patients worldwide, achalasia has the predilection for male sex (61%:39%), and family cases and associated disorders are very rare. Poornachand et al. [1] state that the median time from symptoms onset to diagnosis is nearly three years. The patient's age and the rarity of achalasia in pediatric population are the reasons that our patient's condition was considered as distal esophageal peptic stenosis due to GER and treated by antiulcer therapy. Esophageal manometry is the gold standard, as it shows the elevated basal pressure of LES, the absence of its relaxation on swallowing, and the absence or reduction of esophageal peristalsis [1]. Unfortunately, this procedure was not performed in our patient because of his age. High-resolution impedance manometry can be very useful in the diagnostics [8, 9]. CT scan, contrast studies, and esophagoscopy are very reliable and useful diagnostic methods. Upper GI series, showing dilated, aperistaltic esophagus and narrowed esophagogastric junction as "bird beak" is diagnostic in more than 92% of patients [7]. Our patient's CT, radiography and esophagoscopy findings were characteristic.

The decreased esophageal peristalsis and the absence of LES relaxation cannot be repaired, so the treatment is aimed to reducing LES pressure. Conservative treatment of achalasia is based on the reduction of LES pressure by using anticholinergic and spasmolytic medications. Administration of isosorbide dinitrate, calcium channel blockers and nifedipine showed some positive effects in adults, but not in children [1, 8]. In our patient this kind of medical treatment was not applied because of his age. Intrasphincteric injection of botulinum toxin also does not give satisfactory results [1]. The botulinum injection treatment was not considered as a therapeutical mode in our patient, considering negative results announced in many articles and the child's age. In adult patients, PD can achieve success in 70–80% [10]. Azizkhan et al. [11], however, showed that in pediatric population only 25% of patients responded favorably to repeated PD, while 75% had to undergo a myotomy subsequently, emphasizing that PD was not successful in any child younger than nine years. Jung et al. [4] recorded the higher success rate of PD comparing to Heller operation (55.5%:44.5% after six months, and 65%:40% after 24 months), but they do not recommend PD in children below six years of age. PD of the esophagus was made in our patient because of the suspicion of distal esophageal peptic stenosis. Peroral endoscopic myotomy is a new method that is gradually gaining importance in the treatment of achalasia in children [1, 8, 12]. However, the surgical treatment, based on esophagocardiomyotomy, has proven to be the most effective method for the treatment of achalasia [1]. Esophagocardiomyotomy can be performed by an abdominal or thoracic approach, but the abdominal approach is preferrable because of possibility of performing the antireflux procedure. Myotomy is followed by partial gastric fundoplication in order to reduce the incidence of postoperative GER (from 13% to 7%), although some authors consider it unnecessary [6]. Myotomy with fundoplication have higher success rate comparing to myotomy alone (91%:73%) [7]. The success rate of HE in children below six years of age is 75% at six months, and 83% at 24 months of follow up [2]. Some authors reported the overall success rate of the treatment of achalasia in children, by PD or HE, or both, of 57% after six months, and 64% after 24 months of follow up [7]. Other authors recorded success rate in children of 70–90% after 24 months, similar to the success rate in adults of 80–90% [4]. In our patient, the myotomy was made longitudinally and encompassed about 3 cm of the distal esophagus and 3 cm of the gastric fundus, with mucosal prolapse and without mucosal opening. The length of myotomy was shorter than presented in literature, considering the patient's age. Esophagocardiomyotomy was difficult, due to the scar tissue caused by previous PD. The Dor anterior gastric fundoplication in the length of 2.5 cm was performed in order to prevent postoperative GER, as well as to cover the denuded mucosa. Nowadays, laparoscopic esophagocardiomyotomy, with or without fundoplication, has gained primacy over laparotomic, as an effective method that allows quick recovery, short hospital stay and good definitive outcome [1, 13, 14, 15].

Despite a very low incidence in pediatric population, esophageal achalasia must be considered as a cause of regurgitation, failure to thrive and respiratory problems in infants and children. Once there is a suspicion, the diagnosis can be easily made by esophageal manometry, upper GI series end endoscopic exam. The surgical treatment, consisted of HE and partial wrapping of gastric fundus, is a method of choice in the treatment of children with achalasia.

Informed consent statement: Consent was obtained from the patient's mother for the publication of this report and any accompanying images.

Conflict of interest: None declared.

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Ахалазија једњака код дечака од две године

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САЖЕТАК

Увод Езофагеална ахалазија је неуродегенеративни поремећај покретљивости једњака, који се карактерише неефикасном или одсутном перисталтиком једњака, као и одсуством релаксације хипертоничног доњег езофагусног сфинктера. Ахалазија узрокује застој у напредовању деце и може имати озбиљне респираторне компликације. Ахалазија је веома ретко стање у дечјој популацији и обично се погрешно дијагностикује као гастроезофагусни рефлукс. Метода избора у лечењу је Хелерова езофагокардиомиотомија. Циљ овог рада је да се прикаже редак случај ахалазије код двогодишњег детета, дијагностички поступци и успешно оперативно лечење.

Приказ болесника Тегобе су почеле у узрасту од шест месеци у виду чујног дисања и респираторног стридора. Болесник је примљен у локалну болницу у узрасту од седам месеци, отпуштен са препорукама о исхрани, а поново примљен у узрасту од 19 месеци и преведен у нашу установу. Контрастна радиографија и компјутеризована томографија су показали налазе карактеристичне за ахалазију, а на езофагоскопском прегледу није било отварања доњег езофагусног сфинктера и кардије при инсуфлацији. Учињена је пнеуматска дилатација са привременим побољшањем. Затим је лапаротомијски успешно учињена Хелерова кардиомиотомија са делимичном фундопликацијом по Дору.

Закључак Ахалазија је веома ретко стање код одојчади и мале деце. Често постоји кашњење у постављању тачне дијагнозе. Контрастна радиографија и ендоскопски преглед су најпоузданије методе за постављање дијагнозе ахалазије. Фармаколошки третман, интрасфинктерична инјекција ботулинум токсина и пнеуматска дилатација нису ефикасне методе, нарочито код мале деце. Метода избора у лечењу ахалазије је Хелерова езофагокардиомиотомија са делимичном фундопликацијом.

Кључне речи: ахалазија; деца; хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Drug rash with eosinophilia and systemic symptoms syndrome in an adolescent – efficiency of immunoglobulin G in a corticosteroid-resistant case

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SUMMARY

Introduction Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome (sy) to carbamazepine has a heterogeneous clinical presentation.
The aim of this report is to indicate the efficacy of immunoglobulin G in the treatment of corticosteroid-resistant DRESS sy.

Case outline An adolescent suffering from epilepsy treated with carbamazepine and Na-valproate was hospitalized for fever, elevated transaminases, lymphadenopathy, splenomegaly. There was an eruption of skin efflorescence daily. On the sixth day of hospitalization, the number of eosinophils increased to 24% (780/ml absolute number). There was no desired response to methylprednisolone during the first eight days of treatment or to prednisolone during further treatment, with concomitant administration of antihistamines from day one of hospitalization, to Na-valproate, metformin hydrochloride, elimination diets, and carbamazepine withdrawal. Significant clinical, hematologic, and biochemical improvement occurred the day after the first dose of intravenous immunoglobulin G (IVIG).

Conclusion We point out the need to change the DRESS sy treatment recommendations in favor of the IVIG (as soon as the third or fourth day of treatment) in patients in whom the treatment with corticosteroids has no effect. Until new cases of the proven role of IVIG in the treatment of DRESS sy are published, corticosteroids remain the first therapeutic choice.

Keywords: drug hypersensitivity syndrome; pediatrics; corticosteroids; immunoglobulin G

INTRODUCTION

We are already aware of the mechanisms of the hypersensitive reaction to carbamazepine (IVb/c) in the clinical form named drug rash with eosinophilia and systemic symptoms (DRESS) syndrome (sy) [1, 2, 3]. The heterogeneous clinical presentation of DRESS sy is always a challenge, especially if the administration of carbamazepine for five to six weeks is accompanied by the use of Na-valproate and metformin (previous seven months), all preceded by the infection with herpes simplex virus type 1 (HSV1) [4, 5, 6]. There are two questions in these circumstances: 1) Is the adverse reaction to the drug purely pharmacological (type B), i.e. pharmacological interaction with the immune receptors (p-i), or is it predominantly immune, resulting from the loss of the control mechanism of the immune system due to the previous contact with viruses?; and 2) Why is the efficacy of corticosteroids unsatisfactory so that the successful control of the clinical picture of DRESS sy is achieved with the application of class G immunoglobulins (Ig)? [7, 8, 9].

In this study, we presented a 16-year-old patient whose DRESS sy has been successfully resolved with IVIG.

CASE REPORT

A 16-year-old girl was hospitalized due to the fever up to 38.5°C during the previous day, elevated transaminases, discrete lymphadenopathy, splenomegaly, whereas subjectively in good general condition. In the past few years, the adolescent was treated for Na-valproate epilepsy, but since the seizures were being reported in recent months, carbamazepine was added five to six weeks prior to this hospitalization. Insulin resistance was determined seven months earlier, which is the reason of her regular use of metformin.

During the first seven days of hospitalization, the patient fevered up to 38.5°C mainly in the evening, and there was a daily eruption of skin efflorescence [predominantly in the form of erythematous rash (partly maculopapular) and partly in the form of urticates on the trunk, upper and lower extremities, neck, occasionally on the face), followed by itching, without accompanying bullae, present clinically until the ninth day of hospitalization. Edema of the face manifested itself on the fifth to seventh day of hospitalization. During the first nine days of hospitalization, lymphadenopathy in the neck was pronounced, bilateral, along the sternocleidomastoid and the submandibular muscle, with

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nodules up to 2 cm in diameter, arranged in a row, painless and mobile relative to the base. During this period, the adolescent had normal vital functions, including adequate diuresis, but easily adynamic, which is due to the disease and the use of double doses of antihistamines (cetirizine).

Hematological and biochemical analyzes performed upon admission showed the following: leukopenia (3.9×10^{-9} l) with 21% monocytes and 10% eosinophils (380/ml absolute number), CD4/CD8 ratio was 1031/637/ul (1.62), total CD3 was 1714/ul; high values of transaminase AST was 164 IU/l, ALT was 125 IU/l, gamma-GT was 780 IU/l, lactic dehydrogenases 925 U/l, alkaline phosphatases 553 IU/l, B-type natriuretic peptide 1395 pg/ml, CRP 27 mg/l, and at the same time reduced values of fibrinogen-C (1.9 g/l), activated thromboplastic time of 22.4 seconds, serum IgG concentration of 6.64 g/l, and vitamin D of 10.7 ng/ml. The consumption of IgG in addition to normal serum IgE, IgA, IgM values was followed by the increase in C3 (2.87 g/l) and C4 (0.59 g/l) complement components.

On the fifth day of hospitalization, the number of eosinophils increased to 12% (720/ml absolute number), and on the sixth day of hospitalization to 24% (780/ml absolute number), while at the same time monocytosis was maintained at 17%. Both disorders in blood cells' number were normalized by day 16 of hospitalization. Platelet and erythrocyte counts were always within normal limits. Splenomegaly was pronounced on the ninth day of hospitalization (134×82 mm), and normalized by the 16th day of hospitalization, and hepatomegaly was pronounced on the 16th day of hospitalization (anteroposterior diameter of 160 mm), and normalized by the 24th day of hospitalization.

Serologic testing revealed an elevated IgG antibody titer for HSV1, while within the normal range were the titers of other antibodies (HSV1-IgM, HSV2-IgG and IgM, as well as IgG- and IgM- for Epstein–Barr virus, heterophilic antibodies, cytomegalovirus, *Toxoplasma*, parvoB19, hepatitis A, C, HBsAg, *Mycoplasma pneumoniae*, antistreptolysin titer, lupus antibodies LAC and LAC-SCT). The following biochemical analyses from serum were within the reference ranges: erythrocyte sedimentation, procalcitonin, prothrombin time, international normalized ratio, D-dimer, ferritin, gas analysis, ionogram, glycemia, hemoglobin-A1c, urea, creatinine, proteins, albumin, troponin-hs-I, creatinine kinase, muscle creatinine kinase, valproic acid level, thyroid stimulating hormone, free thyroxine, antinuclear antibodies. No nasal and pharyngeal swab revealed pathogenic germs, nor did the examination of the stool reveal intestinal parasites and *Giardia lamblia*. Urinalysis, occult bleeding stools, lung, and heart X-rays, heart and kidney and pancreatic ultrasound findings, spirometric findings, pulmonary, cardiac, and infectological clinical examinations were all within the reference values. The neurological finding was unchanged compared to the previous period.

DISCUSSION

Significant clinical, hematologic and biochemical improvement occurred the day after the first dose of IVIG, i.e. the IVIG therapy was life-saving in this case [10, 11]. In fact, we started treatment according to RegiSCAR scoring: six out of six criteria for DRESS sy were established and according to this, the treatment was performed with a corticosteroid at a dose of 1 g/kg of body weight (methylprednisolone) intravenously (iv) for eight days with continued antihistamine (cetirizine was administered at twice the regular dose) as well as with the elimination diet, withdrawal of carbamazepine on day sixth of hospitalization, and increased doses of Na-valproate of 1500 mg/day [5]. As the described clinical picture was maintained until the ninth day of hospitalization, and despite the eight days administration of methylprednisolone (iv), the treatment was continued with iv IgG (IVIG, human normal immunoglobulin for intravenous use), even though the indication was not justified by the data from controlled clinical studies but based on case reports [10, 11, 12]. Two doses of IVIG were administered at 0.4 g/kg in eight-day intervals. There was no desired response to methylprednisolone during the first eight days of either treatment nor to prednisolone during further treatment, with concomitant administration of antihistamines from day one of hospitalization, Na-valproate, metformin hydrochloride, elimination diets, and carbamazepine withdrawal. No adverse effects were observed after the administration of two doses of IVIG.

The systemic corticosteroid acts nonspecifically on the tissue by inhibiting the local immune response of the tissue to various stimuli and injuries but does not block the release of mediators, which is the rationale for the ineffective administration of corticosteroids in the present case [13]. However, according to scarce but important literature data, IVIG modulates cytokine production, complement cascades, turnover of B and T cells and neutralization of autoantibodies, thus explaining the effect of treatment and improving the clinical picture the day following administration of the first IVIG dose case [6, 14–17].

Fowler et al.[18] indicated clear gaps in our current understanding of DRESS sy, and pointed to the need for the old test (patch) and new diagnostic tests to screen the patients before starting them on carbamazepine (interleukin-15 and microRNA-122 in the serum), and stated that certain authors favor IVIG and plasma exchange to steroids for the treatment of DRESS sy. Some Korean authors found approximatively 17% of children with adverse skin reactions to antiepileptic drugs (most commonly with aromatic ring in the chemical structure) and significant associations with genes encoding the human leukocyte antigen alleles (e.g. HLA-B*15:02), which indicates the need for genetic testing [19]. The Korean authors published results of the national study and consideration that IVIG monotherapy or the combination of corticosteroids and IVIG might reduce the mortality rate in severe DRESS sy related to antiepileptic drugs [20].

In conclusion, we join the authors who favor the treatment of DRESS sy using IVIG, and we point out the need to change the order of recommendations in the current treatment recommendations for DRESS sy in favor of IVIG [5, 6, 9–18, 20]. IVIG should be considered as a second-line treatment as soon as possible in patients in whom

corticosteroid treatment failed. There is not enough experience for a recommendation that IVIG should be used as the first-line treatment. We advocate for an early introduction of IVIG as early as the third or fourth day from unsuccessful corticosteroid treatment. Further, it is necessary that similar case reports should be collected. Only when new reports on the same topic arrive, corticosteroid treatment shall be considered as second-line, which would have the following consequence – the identification of DRESS sy in children, through pharmacopoeias worldwide, as a proven indication area for IVIG, which is what we advocate. Finally, we invite the professional and scientific community to specify, through subsequent case reports and well-designed controlled clinical studies, the dose of IVIG for the DRESS sy indication area in children, especially caused by carbamazepine, and in light of previous and current viral infections. Unquestionably, the culprit drug should be discontinued immediately.

Informed consent was obtained from the patient's parent prior to her participation in treatment and the parent was informed of the introduction of each drug throughout treatment.

This paper was done in accordance with the institutional committee on ethics.

Conflict of interest: None declared.

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Синдром осипа на лек праћен еозинофилијом и системским симптомима код адолесцента – ефикасност имуноглобулина Г код болесника резистентног на кортикостероид

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САЖЕТАК

Увод Синдром осипа на лек праћен еозинофилијом и системским симптомима (*DRESS*) на карбамазепин има хетерогену клиничку презентацију.

Циљ овог приказа је да укаже на ефикасност имуноглобулина Г у лечењу синдрома *DRESS* резистентног на кортикостероид.

Приказ болесника Адолесценткиња оболела од епилепсије и лечена карбамазепином и натријум-валпроатом хоспитализована је због повишене температуре, повишених трансаминаза, лимфаденопатије, спленомегалије. Сваког дана се испољавала ерупција ефлоресценција на кожи. Шестог дана хоспитализације број еозинофила порастао је на 24% (апсолутни број 780/*ml*). Није било жељеног одговора на метилпреднизолон током првих осам дана лечења нити на преднизолон током даљег лечења, уз истовремену примену антихистаминика од првог дана хоспитализације, натријум-валпроата, метформин-хидрохлорида, елиминационе исхране и укидање карбамазепина. Значајно клиничко, хематолошко и биохемијско побољшање десило се дан после прве дозе интравенског имуноглобулина Г.

Закључак Указујемо на потребу да се измене препоруке за лечење синдрома *DRESS* у корист интравенског имуноглобулина Г (што пре, већ трећи или четврти дан лечења) код болесника код којих је лечење кортикостероидима без ефекта. Све док се не публикују нови случајеви доказане улоге интравенског имуноглобулина Г у лечењу синдрома *DRESS*, кортикостероиди остају први терапијски избор.

Кључне речи: синдром преосетљивости на лекове; педијатрија; кортикостероиди; имуноглобулин Г

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Primary hepatic pregnancy

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SUMMARY
Introduction Hepatic pregnancy is an extremely rare form of ectopic pregnancy, and represents a difficult challenge for both diagnostics and treatment.
Case outline A 40-year-old *gravida* 0 *para* 0 patient in 6 + 0 gestational weeks was admitted to the hospital with lower abdominal pain, positive bHCG values, and presence of free intra-abdominal fluid. She had a history of infertility, and also a previous surgery due to pelvic endometriosis. Urgent open surgery was performed due to signs of hypovolemic shock. We discovered a rupture of the left ovarian corpus luteum cyst. Bleeding management was achieved with preservation of ovarian tissue. Patient recovered, bHCG levels continued to rise, and five days after surgery free intra-abdominal fluid reappeared with upper abdominal pain and tenderness. After transferring patient to abdominal surgery clinic, second surgery was performed, where we confirmed the presence of hepatic pregnancy. After this procedure, patient fully recovered.
Conclusion The method of choice for an ectopic pregnancy treatment is laparoscopic surgery, but when laparoscopy is not possible, the site of ectopic pregnancy could be difficult to diagnose. Prolonged time for making accurate diagnosis increases the risk of ectopic pregnancy complications.
Keywords: hepatic pregnancy; ectopic pregnancy; bHCG; acute abdomen

INTRODUCTION

Ectopic pregnancy is a rare condition that occurs in 1–2% of all reported pregnancies [1]. There has been an increase in the incidence of ectopic pregnancies over the past few decades. This could be explained by three contributing factors: a greater use of assisted reproductive technologies, a high incidence of pelvic inflammatory diseases, and increased awareness of this condition. However, the incidence of abdominal pregnancy is extremely low, and it occurs in only 1% of all ectopic pregnancies [2].

Abdominal pregnancy refers to a pregnancy implanted in the peritoneal cavity, external to the uterine cavity and fallopian tubes. The placenta can be implanted in any of the abdominal organs and it can separate from the site of implantation at any time during pregnancy, which leads to hemorrhagic shock. Abdominal pregnancy is a potentially life-threatening form of ectopic pregnancy because of difficult and often late diagnosis. It is associated with a wide range of signs and symptoms according to the location. The diagnosis of abdominal pregnancy is often missed during routine ultrasonography (US) [3].

In contrast to tubal ectopic pregnancies, primary methotrexate therapy of early gestations in abdominal pregnancy has a high risk of failure due to more advanced gestational age at which these pregnancies are discovered [4].

CASE REPORT

A 40-year-old patient was admitted to hospital with lower abdominal pain. She had a history of infertility and had a conservative surgery due to endometriosis (done five years prior through lower transverse laparotomy). Amenorrhea was 6 + 0 weeks. The initial value of β-HCG was 621 mIU/mL. US exam showed the presence of intra-abdominal free fluid and empty uterine cavity with no adnexal masses. Monitoring of vital signs showed systolic blood pressure below 90, pulse rate of 130 bpm, and diuresis below 30 ml/h (after receiving 500 ml of 0.9% Sodium Chloride solution intravenously). Due to clinical findings of intra-abdominal bleeding and hypovolemic shock, the patient underwent an emergent surgical procedure by lower transverse laparotomy approach from previous surgery. Intraoperative finding was the rupture of the left ovarian corpus luteum cyst with heavy intra-abdominal bleeding (over two liters). Both Fallopian tubes and the right ovary were unaffected. Small bowels, caecum, rectosigmoid colon, and infracolic part of omentum were explored and no sign of ectopic pregnancy was noticed. Histopathological report showed a corpus luteum cyst on the left ovary. After the surgery, the patient fully recovered, but β-HCG levels continued to rise (1052 mIU/mL). Postoperative US showed no signs of either intrauterine or extrauterine pregnancy. At that time, a small formation found in the liver, 37 × 25 mm in diameter, was considered clinically indistinctive (Figure 1).

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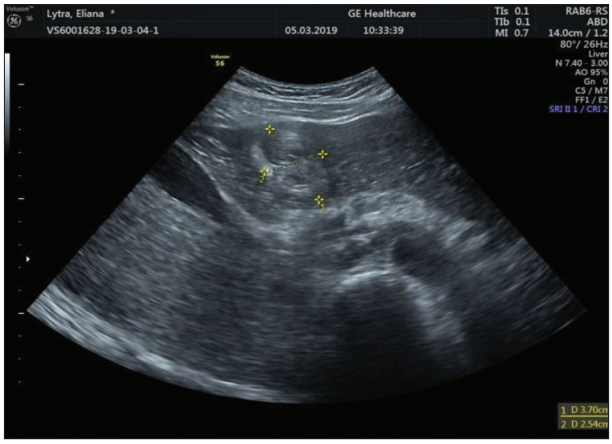

Figure 1. Ultrasonography finding: at first no significance was given to formation found in liver near hilus; differential diagnosis was liver hemangioma

All clinical findings suggested the presence of ectopic pregnancy in the liver. The patient was transported to abdominal surgery clinic for further evaluation and treatment. An urgent computed tomography revealed the presence of a mass of 6 × 5 cm in diameter in the left liver lobe (between liver segments III and IV), with an oval central portion of lower density, and with the peripheral region of higher density in the contrast plan scan. There was a significant amount of fluid, which corresponds to the density of blood, below the left lobe of the liver and above the stomach (Figure 2).

After the urgent computed tomography examination, the patient underwent an open surgery with an upper medial laparotomy. We found around 500 ml of blood in the space between the left lobe of the liver and the stomach. After the evacuation of a blood which was predominantly coagulated,

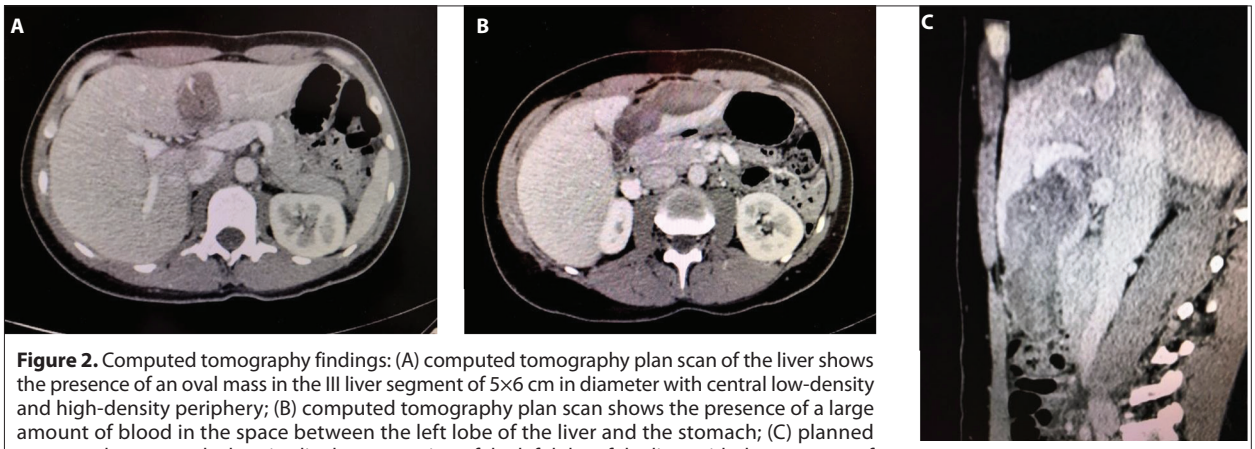

Figure 2. Computed tomography findings: (A) computed tomography plan scan of the liver shows the presence of an oval mass in the III liver segment of 5×6 cm in diameter with central low-density and high-density periphery; (B) computed tomography plan scan shows the presence of a large amount of blood in the space between the left lobe of the liver and the stomach; (C) planned computed tomography longitudinal cross-section of the left lobe of the liver with the presence of blood in the space between the liver and the stomach

On the third postoperative day we performed diagnostic dilatation and curettage of the uterine cavity. Histopathological exam indicated the presence of an endometrial intraepithelial neoplasia, but no evidence of intrauterine pregnancy. After this intervention β-HCG levels dropped to 861 mIU/mL, but soon continued to rise to the level of 1132 mIU/mL. Since intrauterine pregnancy was excluded, we suspected the presence of ectopic abdominal pregnancy of unknown location and administered a single dose of methotrexate (50 mg).

The patient decided to leave the hospital against medical advice. She was suggested to repeat serum β-HCG values in three days. The values were higher than previous, 1668 mIU/mL. On the fifth day upon methotrexate administration, the patient checked into our hospital with pain in the upper parts of the abdomen. Levels of β-HCG were 2060 mIU/mL. On US we discovered the presence of free fluid intra-abdominally (small quantities) and abnormal US imaging of the liver. The US exam revealed a hyperechogenic structure of six centimeters in diameter in the left lobe near the hilum of the liver. The patient had tenderness in the upper parts of the abdomen.

ectopic pregnancy was detected below the falciform ligament in the third liver segment. Expulsion of the ectopic pregnancy was then performed from the existing liver cavity. Gauze with hypertonic solution of sodium chloride was placed in the cavity of the ectopic pregnancy. The surgical procedure was completed with a single liver suture, as well as hemostasis with a bipolar diathermy. More than three liters of isotonic solution of sodium chloride was placed in the abdominal cavity and two drains were placed: one under the liver, and the other in Douglas space (Figure 3).

The β-HCG value (460 mIU/mL) one day after the surgical procedure indicates a significantly lower value than before the surgery. Six days after the surgery the β-HCG level was 32 mIU/mL. Prior to the surgical procedure, hemoglobin value was 109 g/L, and on the day of the patient`s release it was 110 g/L only with one blood transfusion (280 ml) after the operation. The patient was discharged on the seventh day in excellent condition.

The histopathology exam confirmed diagnosis of ectopic pregnancy with presence of trophoblastic tissue found in liver.

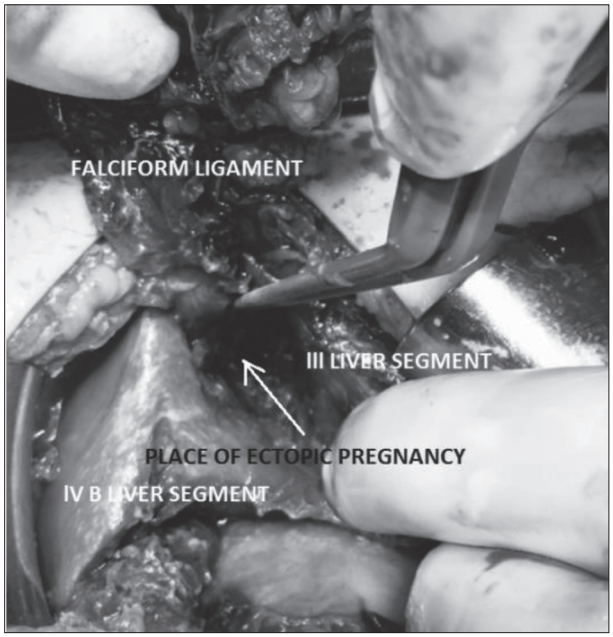

Figure 3. Intraoperative findings: segment III of the liver and place of the ectopic pregnancy next to segment IV of the liver below the falciform ligament after the expulsion of the ectopic pregnancy from the liver cavity

DISCUSSION

Ectopic liver pregnancy is a very rare condition (1:15,000 pregnancies), but also a very dangerous gynecological disease mostly because of life-threatening intra-abdominal bleeding especially when occurring in the liver or spleen [5, 6]. Although the mechanism of primary hepatic pregnancy is unknown, it can be assumed that the risk factors of its occurrence could be: ovarian cysts, adhesions, pelvic or tubal inflammatory diseases, as well as the specificity of the hepatic tissue characterized by huge blood supply, which could lead to a higher possibility of trophoblast implantation and development of gestation sac [7].

In the presented case, the patient had previously suffered from endometriosis and an ovarian cyst, which have been proven operatively and by ultrasound examination. In our case, the rise of β-HCG levels after the prior operation was an alarm for ectopic pregnancy progression.

Further evaluation of endometrial intraepithelial neoplasia found after dilation and curettage procedure is required to confirm the diagnosis of a true premalignant endometrial lesion and exclude an associated endometrial carcinoma in the patient [8]. Inadequately prepared, as well as pathologically altered endometrium could be one of the explanations for ectopic pregnancy in the presented case.

Abdominal pregnancy represents great diagnostic challenge. A common symptom for hepatic pregnancy is upper right abdominal tenderness. However, our patient did not show any symptoms regarding upper parts of abdomen in the beginning, which made diagnostic process more difficult [3, 9].

It is important to emphasize the presence of two episodes of internal bleeding in this case. The reason for the first episode of bleeding was a rupture of the left ovarian corpus luteum cyst, while the second episode occurred due to the bleeding from the liver at the implantation site of trophoblastic tissue.

Preferred method of choice for treatment of ectopic pregnancy should be laparoscopy, where whole abdominal cavity could be explored [10, 11]. Certain, uncomplicated cases of hepatic pregnancy could be treated with methotrexate [9]. In cases of hepatic pregnancy medial laparotomy could have advantage in bleeding control, considering vascularization of the hepatic tissue [12]. Unfortunately, due to hypovolemic shock, our patient underwent urgent open surgery. Laparoscopy should be performed whenever possible to avoid misdiagnosis of abdominal pregnancy and further complications.

NOTE

Ethical compliance statement: We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

Ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material has been obtained from the patient.

Conflict of interest: None declared.

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Примарна хепатична трудноћа

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САЖЕТАК

Увод Хепатичка трудноћа је веома редак облик ектопичне трудноће и представља велики изазов за дијагностику и лечење.

Приказ болесника Четрдесетогодишња болесница (*para* 0, *gravida* 0), у 6 + 0 недељи трудноће примљена је на клинику због болова у доњем делу абдомена, са позитивним вредностима *bHCG* и слободном течношћу у абдомену. У анамнези се наводи присутан инфертилитет, као и претходна операција због ендометриозе. Због знакова хиповолемијског шока, болесница је хитно оперисана. Интраоперативно је констатована руптура цисте жутог тела левог јајника. Хемостаза је успостављена уз очување ткива јајника. Болесница се опоравила, али су вредности *bHCG* наставиле да расту и петог постоперативног дана долази до поновног накупљања слободне течности у трбуху са појавом болова и напетости у горњим делу абдомена. После превођења болеснице на одељење абдоминалне хирургије, поново је оперисана и том приликом потврђено је да се ради о хепатичној трудноћи. После ове процедуре болесница се потпуно опоравила.

Закључак Метода избора за ектопичну трудноћу је лапароскопска хирургија, али када лапароскопија није могућа, место ектопичне трудноће није лако пронаћи. Продужено време у постављању прецизне дијагнозе повећава ризик од компликација ектопичне трудноће.

Кључне речи: хепатична трудноћа; ектопична трудноћа; *bHCH*; акутни абдомен

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Fractures of the acetabulum – surgical treatment and complications

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SUMMARY
Acetabular fractures represent severe injuries that mostly occur in car accidents, or after falling from greater heights, most often in the working male population. Acetabular fractures are present in our clinical practice and their treatment requires good education and surgical training. Surgical experience is one of the prerequisites for achieving good treatment results, since these fractures are accompanied by numerous complications. In order to acquire knowledge and skills in this field of surgery, it is necessary to have a national center for education at one of the medical faculties in Serbia. All dislocated acetabular fractures (≥ 2 mm) require early surgery, anatomical reduction, and stable internal fixation of acetabular fracture. Acetabular fracture-dislocation requires urgent reduction of the dislocated femoral head. The anatomic reduction of the fracture is related to the time of definitive bone fixation of the fracture. Fourteen days after the fracture, anatomic reduction is more difficult to achieve. In addition to the factors that positively affect the results of treatment, there are negative factors as well, which result in poor outcomes. They are directly correlated to the initial trauma that occurs at the time of injury. Fracture comminution, large dislocation (> 20 mm), injury of the femoral head, posterior dislocation of the hip, impaction, traumatic or iatrogenic sciatic nerve palsy – these are all factors that negatively affect the outcome and are responsible for complications, as opposed to positive factors.
Keywords: acetabulum; fractures; surgical treatment; complications

INTRODUCTION

The poor outcomes of conservative treatment of acetabular fractures, back in the 1950s, led Letournel and Judet [1] to embark on a new era of surgical treatment. The principles of open reduction and stable internal fixation that they founded are still valid today, despite the great advances in orthopedics and traumatology. Acetabular fractures are severe, occurring in young, working, more frequently male population, in car accidents or in falls from heights [2]. The incidence of acetabular fractures is about three fractures per 100,000 patients per year [3]. The city of Niš is the largest city of the Nišava District with a population of about 350,000, over 2,000,000 inhabitants of Southern and Eastern Serbia gravitate towards it. It has a tertiary institution and an incidence of acetabular fractures of about three fractures per 100,000 patients per year. Considering the gravitational and treatable population at the Clinical Centre of Niš, the Clinic for Orthopaedic Surgery and Traumatology has made a significant step forward with regard to the modern approach and treatment of acetabular fractures. In younger patients, these fractures are usually caused by a strong axial force acting through the femoral shaft or a direct force acting through a greater trochanter. In the elderly, acetabular fractures can cause low-energy trauma due to the presence of osteoporosis. Acetabular fractures, primarily dislocated (> 2 mm), are treated surgically with open fracture reduction and stable internal fixation with acetabulum reconstructive plates/screws. The complications that accompany these fractures are numerous – traumatic sciatic nerve injury, iatrogenic sciatic nerve injury, infection, revision osteosynthesis, deep vein thrombosis (DVT), heterotopic ossification (HO) – Broker I–IV, femoral head osteonecrosis, secondary osteoarthritis (OA) of the hip [4]. Some of these complications require later revision surgery, which is reflected in total hip replacement [5]. Due to all of the above and the complexity of acetabular surgery, constant education of the surgeon and surgical experience are required to achieve excellent and good outcomes, as it has been shown that surgical experience is an important factor directly correlated to achieving excellent and good outcomes [6].

CLINICAL ANATOMY OF THE ACETABULUM

The clinical anatomy of the acetabulum divides the acetabulum into the anterior and posterior columns, which are arranged in the inverted "Y" shape. The anterior column is the anterior part of the iliac bone that extends to the pubic bone. It contains the anterior part and the edge of the iliac wing, the pelvic edge, the anterior wall of the acetabulum, and the upper branch of the pubic bone. The posterior column consists

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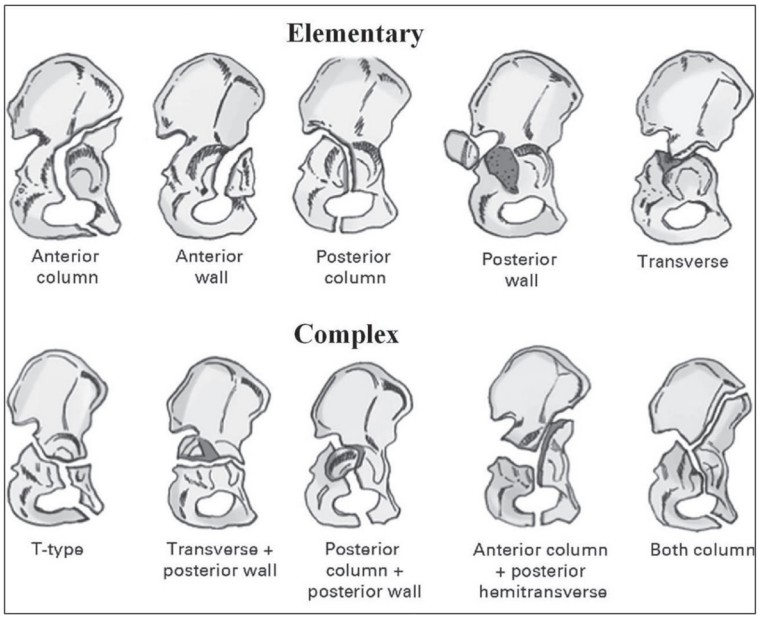

Figure 1. Classification of acetabular fractures according to Letournel and Judet [9]

of parts of the iliac and ischiadic bones, large and small ischiadic notches, posterior wall of the acetabulum, most of the quadrilateral surface, and ischiadic tuberositas. The upper part of the acetabulum, through which load forces are transmitted, is called the roof of the acetabulum. The vertical line that runs through the center of the femoral head and the line that goes through the fracture of the acetabulum make an angle called the "acetabular roof angle" [4].

MECHANISM OF INJURY

Acetabular fractures are caused by the action of an axial force through the femoral shaft. The type of fracture of the acetabulum, its anterior or posterior structure, depends on the position of the femoral head at the time of impact into the acetabulum. Another way of creating an acetabular fracture is through the action of a direct force over a greater trochanter when the quadrilateral surface of the acetabulum (central luxation) is most commonly fractured [4].

CLASSIFICATION OF ACETABULAR FRACTURES

The pioneers of acetabular surgery, Letournel and Judet [1], represented a classification that stood the test of time, and is still valid and applicable worldwide. According to this classification, acetabular fractures are divided into elementary and complex [7, 8, 9] (Figure 1).

RADIOLOGIC EVALUATION

Our teachers, our teachers' teachers used clinical examination and radiographic diagnostics, which included radiography in the antero-posterior position and two oblique Judet views (iliac oblique and obturator oblique). These three projections were sufficient for the experienced surgeon to evaluate the stability of the fracture and determine the surgical approach during surgical treatment. Modern diagnostics in the form of computed tomography (CT) and 3D-CT allows the surgeon to see a clear three-dimensional image of the acetabulum that will determine the type of surgical approach, will allow him to see the size of the bone fragments, the degree of dislocation, comminution, impaction, the presence of loose bodies in the acetabulum [10, 11].

TREATMENT OF ACETABULAR FRACTURE

Undislocated (≤ 2 mm), stable acetabular fractures can be treated conservatively. The question is whether skeletal traction is required in this treatment. The authors believe that skeletal traction is not necessary in undislocated acetabular fractures; the patient can walk without weight-bearing for six to eight weeks. Partial to full weight-bearing is allowed after this period, with rehabilitation. In patients with dislocated fractures who cannot undergo surgical treatment, closed reduction via skeletal traction with bed rest for the initial six to eight weeks may be used. Dislocated (≥ 2 mm) and unstable acetabular fractures are treated surgically – by open reduction and stable internal fixation, or by percutaneous minimally invasive surgery, which require experience and intraoperative fluoroscopy. In order to achieve satisfactory functional and radiographic results, it is necessary to achieve acetabular congruence and anatomic reduction, stable internal fixation. Early activation and rehabilitation is required, without weight-bearing from six to eight weeks after surgery, when partial weight-bearing begins to increase and progressively increases over the next few weeks, until full weight-bearing is achieved [6, 12, 13, 14]. The most common surgical approaches used for surgical open reduction and internal fixation are anterior ilio-inguinal, anterior ilio-femoral, posterior Kocher–Langenbeck, combined anterior and posterior, modified Stoppa, anterior pararectal surgical approach (Figures 2–5). Understanding of these surgical approaches requires training, continuous education and raises the question of the existence of a national educational center, because, regardless of the number of orthopedic surgeons, there are very few who are familiar with this pathology.

PRIMARY TOTAL HIP REPLACEMENT AFTER ACETABULAR FRACTURE

There is much controversy regarding primary total hip replacement in fresh acetabular fractures. The issue of "fixed or replaced" is always the question, especially in older patients. In any case, primary total hip replacement

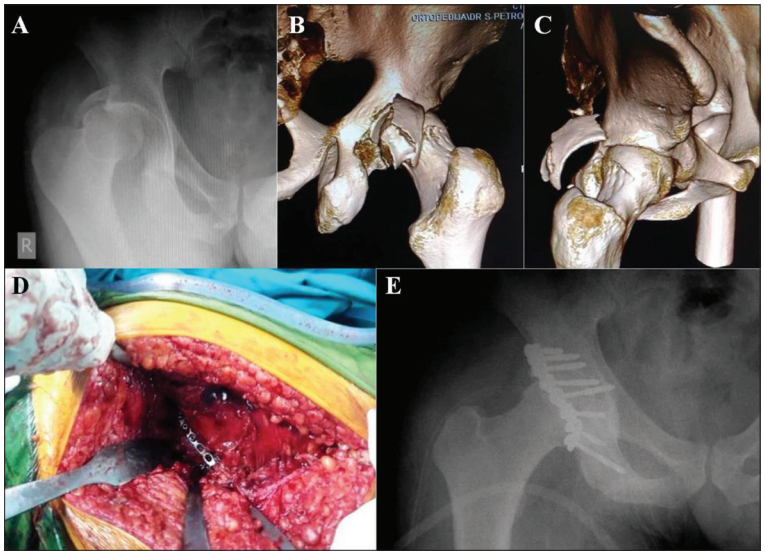

Figure 2. Open reduction and internal fixation of acetabular fracture-dislocation; A: X-ray after the injury; B, C: 3D computed tomography scan after reduction of dislocated femoral head shows a dislocated posterior wall acetabular fracture; D: intraoperative view after fracture fixation by Kocher–Langenbeck surgical approach; E: X-ray after the surgery

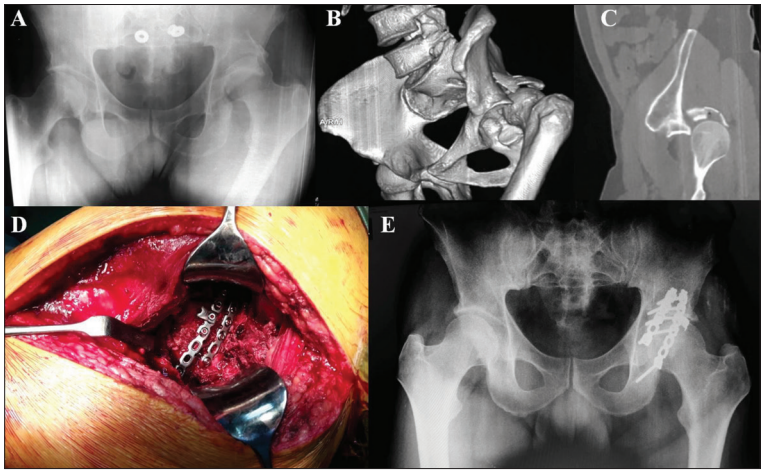

Figure 3. Open reduction and stable internal fixation of acetabular fracture-dislocation; A: X-ray after the injury; B: 3D computed tomography (CT) view shows fracture of the posterior wall of the acetabulum and posterior hip dislocation; C: sagittal CT view shows posterior acetabular fracture-dislocation; D: intraoperative view after fracture fixation; E: postoperative X-ray

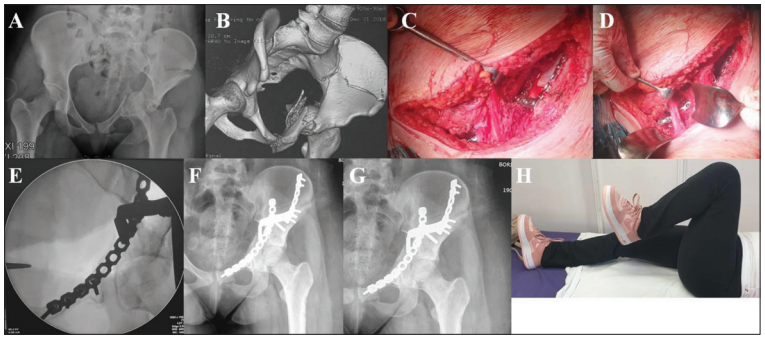

Figure 4. T-fracture of the acetabulum associated with iliac bone fracture in a 20-year-old patient; A: X-ray after the injury; B: 3D computed tomography view; C, D: intraoperative views after fracture fixation through the anterior ilio-inguinal surgical approach; E: intraoperative fluoroscopy; F: postoperative X-ray; G: X-ray six months after the injury; H: functional outcome, after six months post-injury was excellent

is used in the treatment of fresh acetabular fractures, and numerous complications that accompany this surgery are described. Indications are set on a case-by-case basis and recommended for individually selected cases [15, 16, 17] (Figure 6).

COMPLICATIONS AFTER ACETABULAR SURGERY

Based on clinical practice and contemporary literature, the most common complications accompanying the surgical treatment of acetabular fractures are the following: traumatic and iatrogenic sciatic nerve palsy, thromboembolic complications (DVT) and pulmonary thromboembolism (PE), infection, loss of osteosynthesis after surgical fixation of the fracture, HO, femoral head osteonecrosis, secondary OA of the hip [4, 6, 18, 19].

TRAUMATIC AND IATROGENIC SCIATIC NERVE PALSY

Contemporary literature describes traumatic and iatrogenic sciatic nerve palsy or its peroneal division [20, 21]. The injuries of the peroneal division of the sciatic nerve are most common. These injuries are more common in the posterior hip dislocation associated with acetabular fracture, caused by the pressure of the dislocated femoral head or the pressure of the bone fragment from the posterior wall of the acetabulum at the time of injury. According to Bogdan et al. [22], out of 137 patients with acetabular fractures, 57% had traumatic nerve injury. Immediate reduction of dislocated hip and early fixation of the acetabulum reduce pressure on the nerve and allow better functional recovery of the nerve. In addition to the traumatic lesion, iatrogenic injuries to the sciatic nerve have also been described. Iatrogenic injury can be caused by rough surgical work, manipulations during surgery, careless handling of elevators and retractors, the presence of a postoperative hematoma. In order to prevent iatrogenic injury to the sciatic nerve, knee flexion during surgery is necessary to relieve the nerve, clear identification and protection of the nerve during surgery, special attention should be paid to the presence of possible anatomic variations of the sciatic nerve (Figure 7), postoperative drainage is required. Haidukewych et al. [23] reported an incidence of 7.9% of iatrogenic sciatic nerve injuries after acetabular surgery.

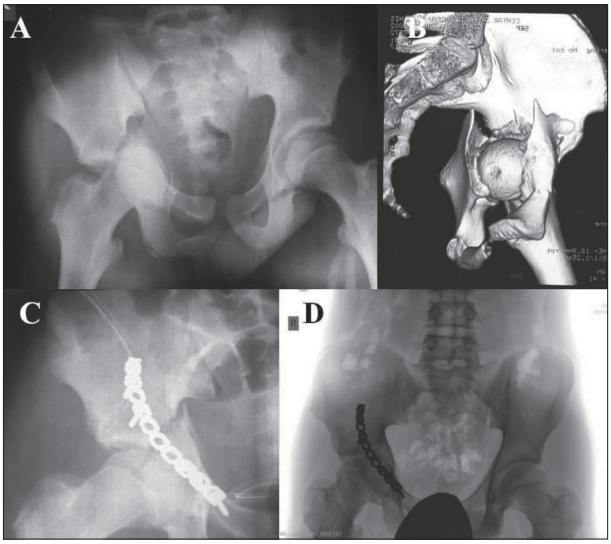

Figure 5. T-fracture of the acetabulum in a 14-year-old patient; in such fractures, surgical reduction and fracture fixation is usually performed with a combined anterior and posterior Kocher–Langenbeck approach in one act or staging surgery at intervals of two to three days; given the patient's age and fracture reduction achieved, we used only anterior approach and further treatment was continued with cutaneous traction for three weeks; A: X-ray after the injury; B: 3D computed tomography view after the injury; C: X-ray after fracture fixation through the anterior ilio-inguinal approach; D: X-ray after six months

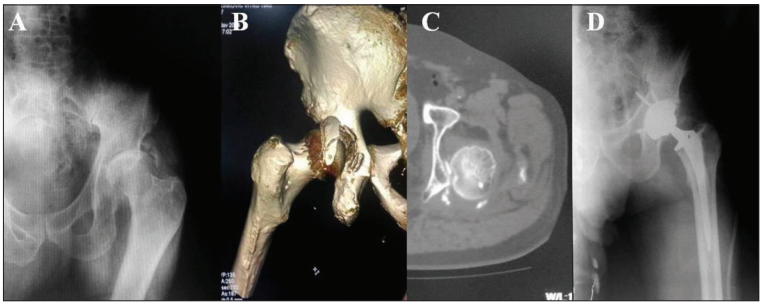

Figure 6. Primary total hip replacement after fresh posterior wall acetabular fracture with posterior hip dislocation in a 74-year-old patient; A: X-ray after the injury; B: 3D computed tomography (CT) view; C: sagittal CT view shows posterior hip dislocation with a fracture of the posterior wall of the acetabulum; D: X-ray after primary total hip replacement

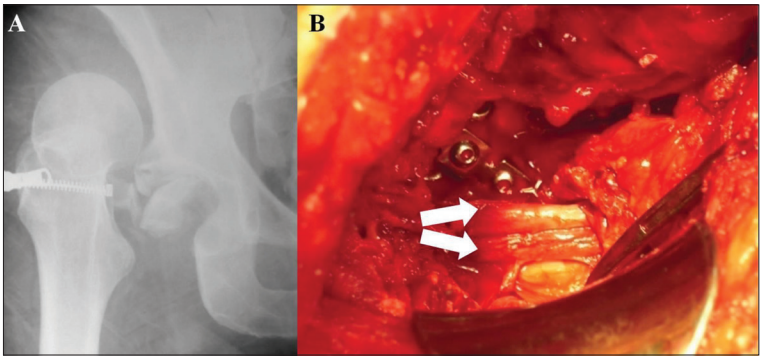

Figure 7. Anatomical variation of the sciatic nerve shows a sciatic nerve high division in the gluteal region, in a 48-year-old patient with a comminuted posterior wall acetabular fracture associated with posterior hip dislocation and traumatic palsy of sciatic nerve; A: X-ray after the injury; B: intraoperative view after acetabular fracture fixation; the arrows show the sciatic nerve high division

THROMBOEMBOLIC COMPLICATIONS (DVT) AND PULMONARY THROMBOEMBOLISM

Post-traumatic and postoperative thromboembolism is a significant problem in patients with acetabular fractures. These complications accompany acetabular surgery despite thromboprophylaxis, especially in elderly patients over 60 years of age, patients with increased risk for DVT, complex fractures, and delayed osteosynthesis of acetabular fractures after two weeks [24]. According to Wang et al. [25], in a series of 110 patients with pelvic and acetabular fractures, 29.09% had DVT, three patients had PE. In addition, the incidence of DVT in patients with acetabular fractures was significantly higher than that of patients with pelvic fractures. According to Althuwaykh et al. [26], the incidence in a series of 404 patients with acetabular fracture was 5%, while 1.7% of the patients had PE. Despite the prophylaxis, the prevalence of post-traumatic and postoperative thromboembolism is approximately 11% [27].

INFECTIONS AND REVISION SURGERY

Early revision surgery is rarely used in cases of loss of fixation or surgical debridement and irrigation in early infections after osteosynthesis of acetabular fractures. Infections, superficial or deep, are rare due to good vascularization but are present and should be considered. Postoperatively, antibiotic prophylaxis is required until postoperative drainage is extracted. According to Ding et al. [28], 7% of patients required revision surgery due to debridement and irrigation after wound infection; according to Iqbal et al. [29], 5.4% required revision. Similar data was reported by Suzuki et al. [30]. According to Negrin and Seligson [31], revision surgery due to secondary loss of reduction, seroma/hematoma, and wound infection was in 6%. According to Giannoudis et al. [32], the incidence of infection after surgical treatment of acetabular fractures was 4.4%.

HETEROTOPIC OSSIFICATION

HO is also clearly described and it accompanies this type of surgery [33]. In many centers, indomethacin or low-dose radiotherapy is administered as prophylaxis to prevent the development of HO [34]. In a meta-analysis of 2394 displaced fractures by Giannoudis et al. [32], the HO incidence was 25.6% with Brooker grade III or IV at 5.7%.

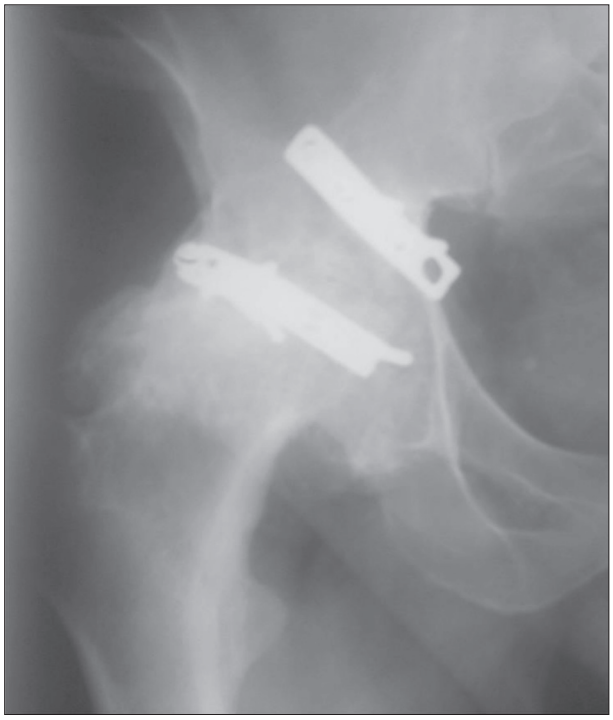

Figure 8. Hip ankylosis in a 73-year-old patient, caused by secondary osteoarthritis of the hip; the acetabular surgery was done at another institution 39 years previously

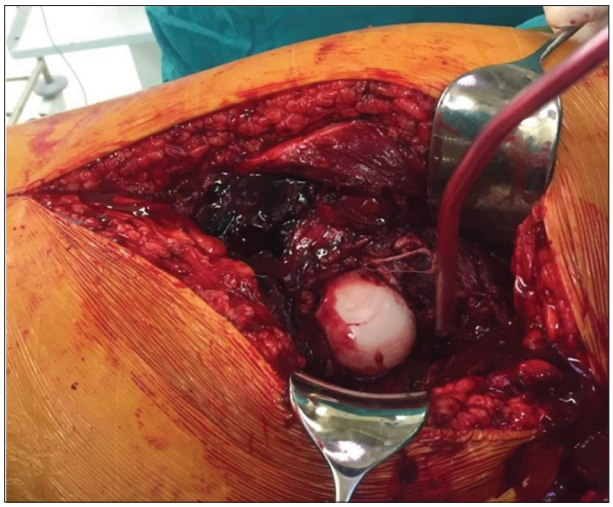

Figure 9. Intraoperative view during the open reduction of a dislocated hip in a 55-year-old patient with the posterior wall acetabular fracture associated with posterior hip dislocation

FEMORAL HEAD OSTEONECROSIS

This complication can occur several months to several years after acetabular fracture. As a result of the femoral head osteonecrosis, fragmentation and collapse of the femoral head can occur, which will cause secondary OA of the hip. Although it is sometimes difficult to diagnostically differentiate the OA and osteonecrosis, it is not uncommon to see both intraoperatively. Different authors describe the different incidence of the femoral head osteonecrosis. According to Pavelka et al. [35], 11.7% of patients developed the femoral head osteonecrosis. The fact is that the femoral head osteonecrosis is much more common in acetabular fractures that are associated with posterior hip dislocation [36]. According to Giannoudis et al. [32], the incidence of osteonecrosis was 5.6%, while the incidence of osteonecrosis after acetabular fracture was 5%, and 9.2% for acetabular fractures associated with posterior hip dislocation. Posterior hip dislocation is an orthopedic emergency and therefore any dislocated hip should be reduced urgently after hospitalization. A number of authors show the importance of urgent reduction of the dislocated hip in the prevention of the femoral head osteonecrosis [37–40]. Late reduction after 24 hours from the injury increases the possibility of osteonecrosis. According to one of our studies, the incidence of the femoral head osteonecrosis after acetabular fracture – dislocations in which the hip was reduced within 24 hours of injury – was 5.55%, while in a hip reduced after 24 hours after the injury occurred, osteonecrosis incidence was 27.77% [41].

SECONDARY OSTEOARTHRITIS OF THE HIP

The occurrence of secondary OA of the hip is associated with a non-anatomical reduction of the acetabular fracture during definitive fixation. The literature describes a significantly lower percentage of secondary OA of the hip in anatomically reduced acetabular fractures [42]. Secondary OA of the hip accompanies acetabular fractures and is usually associated with non-anatomical fracture reduction. Meena et al. [43] published a paper accordin to which not achieving anatomical reduction, associated injuries, initial fracture dislocation (> 20 mm), posterior hip dislocation, late definitive fixation of acetabulum, age, can negatively affect the achievement of good outcome. According to Matta [44], the number of anatomic reductions decreased as time to surgery increased. Pascarella et al. [40] also describe the importance of anatomic reduction of acetabular fractures in achieving excellent and good outcomes. Pavelka et al. [35] published data on 32.81% secondary OA of the hip, 24 months after acetabular fracture. Cahueque et al. [45] published 48% secondary OA, two years after the acetabular fracture. There are other authors who believe that secondary OA occurs several years after the injury, despite anatomic reduction, which only confirms the importance and severity of the acetabular fracture and the anatomical specificity of the acetabulum and hip joint [46] (Figure 8). Some of the cases with secondary OA of the hip require further surgery – total hip replacement [5, 47].

TIME OF DEFINITIVE ACETABULAR FIXATION

Numerous authors agree that the time interval from injury to definitive acetabular fixation should not be longer than seven days, preferably three to five days. Dailey et al. [42] achieved the best anatomic reduction of acetabular fracture in the first three days after the fracture. According to Brueton [48], the timing of surgery was found to be directly related to the quality of the clinical result. Similar results are presented by Matta et al. [44]. With the delay of

definitive acetabular surgery, the possibility of anatomic reduction is reduced. Definitive osteosynthesis after two to three weeks of the fracture impairs fracture reduction, increases intraoperative bleeding, which adversely affects surgical work. In clinical practice, there are also individual cases with acetabular fracture associated with posterior hip dislocation when definitive acetabular fixation is performed within 24 hours after the injury, due to the need for open reduction of the hip that could not have been reduced by the closed method (Figure 9).

SURGICAL EXPERIENCE

Surgical experience, reflected primarily in the manual ability and familiarity of the surgeon with a certain surgical problem, is an important prerequisite for success. In acetabular surgery, surgical experience is of great importance. Surgical experience is one of the preconditions for successful treatment of acetabular fractures. In order to acquire knowledge and necessary skills in this field of traumatology, it is necessary to have a national educational center at one of the medical faculties in Serbia. The literature clearly indicates the importance of surgical experience in the treatment of acetabular fractures [7]. Even though we have a sufficient number of orthopedic surgeons in Serbia, we unfortunately have a small number of surgeons who are experienced in this field of traumatology. So far, this experience has been gained abroad in large trauma centers under the guidance of experts. Although rare, acetabular fractures are present in our traumatology practice. It matters whether the patient will return to pre-operative activity after the acetabular fracture, or whether the acetabular fracture will leave lasting consequences and disability.

CONCLUSION

Proper diagnosis of acetabular fractures, good knowledge of the acetabular anatomy, experience of the surgeon, early definitive acetabular osteosynthesis, anatomic reduction, and early rehabilitation are only prerequisites for achieving excellent and good outcomes. Whether we will have excellent or good outcomes depends on the initial trauma that caused the damage. Damage is often inevitable, and whether it will be less or greater, it may also depend on ourselves, who deal with this segment of traumatology. We have achieved a lot in acetabular surgery, but still not enough to say that we are in step with the developed world. Including more surgeons in our institutions, who will deal with acetabular surgery, education and training, the existence of a national educational center that will have the opportunity to educate on cadavers are necessary if we want to advance this demanding area of traumatology – pelvic and acetabular surgery.

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Преломи зглобне чашице кука – хируршко лечење и компликације

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САЖЕТАК

Преломи зглобне чашице кука представљају изузетно тешке повреде које најчешће настају у саобраћајним удесима или приликом пада са већих висина, најчешће код радно активне мушке популације. Преломи зглобне чашице кука су присутни у нашој клиничкој пракси и захтевају добру едукацију и обученост кадрова за лечење. Хируршко искуство је један од предуслова за постизање добрих резултата лечења јер ове преломе прате бројне компликације. Ради стицања знања и вештина из ове области хирургије, потребно је да постоји национални центар за едукацију при неком од медицинских факултета у Србији. Сви дислоцирани преломи (≥ 2 *mm*) зглобне чашице кука се лече хируршки, а за постизање добрих резултата неопходна је рана анатомска репозиција и стабилна унутрашња фиксација. Код прелома зглобне чашице са ишчашењем кука неопходна је хитна репозиција ишчашеног кука. Анатомска репозиција прелома је повезана са временом дефинитивне коштане фиксације прелома. После 14 дана од прелома анатомска репозиција се теже постиже. Поред ових фактора који позитивно утичу на крајње резултате лечења, са друге стране постоје и негативни фактори који утичу на постизање лоших резултата лечења. Они су директно повезани са тежином иницијалне повреде која настаје у тренутку прелома. Коминуција прелома, велика дислокација (> 20 *mm*), повреда главе фемура, ишчашење кука, утиснуће, трауматска или јатрогена повреда седалног нерва су фактори који негативно утичу на резултате и одговорни су за компликације, насупрот позитивним факторима.

Кључне речи: зглобна чашица кука; преломи; хируршко лечење; компликације

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 и 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 и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор 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 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
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11000 Београд
Србија

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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

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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.

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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.

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