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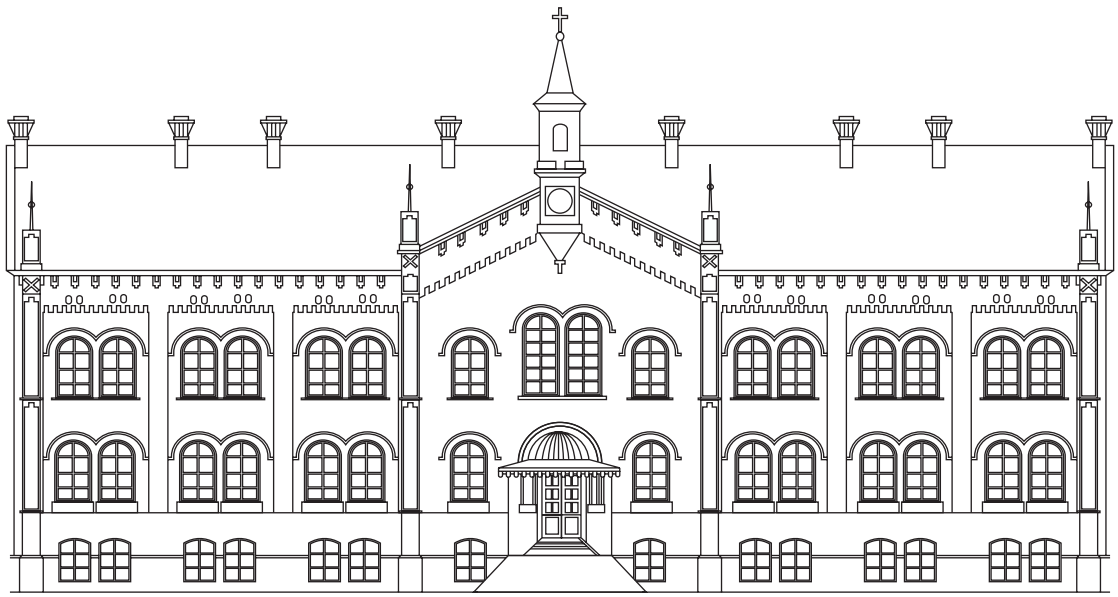

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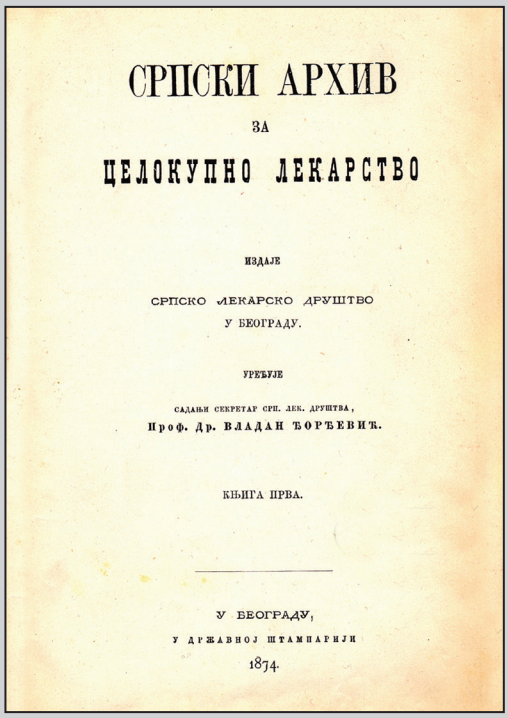

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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У БЕОГРАДУ.

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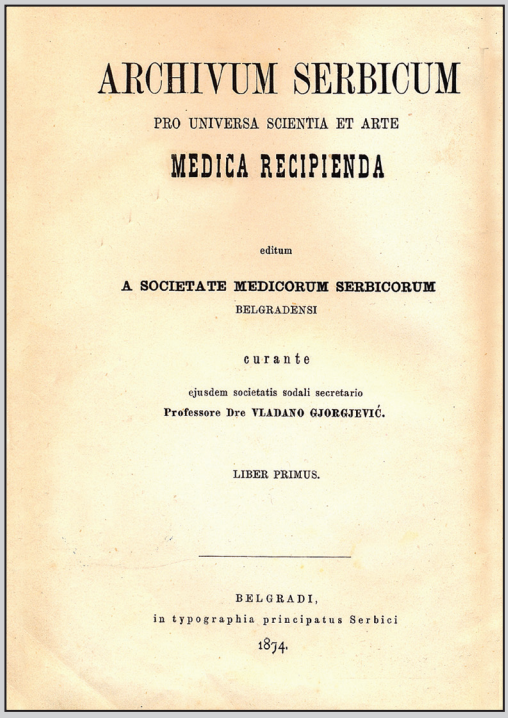
У БЕОГРАДУ,

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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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Endodontic treatment of traumatized teeth with chronic periapical lesions using antibiotic paste and mineral trioxide aggregate obturation – a preliminary study

Bojana Ćetenović[1], Dejan Marković[2], James Gutmann[3], Tamara Perić[2], Vukoman Jokanović[1]

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SUMMARY

Introduction/Objective The purpose of this study was to assess effectiveness of endodontic root canal procedures in traumatized permanent teeth with necrotic pulps and chronic periapical lesions after definitive obturation with mineral trioxide aggregate (MTA) products. Adobe Photoshop CS (San Jose, CA, USA) image-analysis software was used for healing assessment.

Methods Twenty-seven traumatized single-rooted permanent teeth diagnosed with necrotic pulps and chronic periapical lesions were treated with non-surgical procedure using a tri-antibiotic paste and calcium hydroxide as intra-canal medication. Definitive obturation was performed with ProRoot MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) or MTA$^+$ Cerkamed (Cerkamed, Stalowa Wola, Poland). Control follow-ups were done three, six, 12, and 24 months following the completion of treatment.

Results The positive clinical outcome was recorded in 24 (88.9%) cases, while radiographic success was present in 26 (96.3%) cases. A statistically significant decrease in the sizes of periapical lesions was consistently observed at follow-up periods ($p < 0.001$). There was no statistically significant difference between the two tested MTA materials ($p > 0.05$).

Conclusion The MTA products were effective for the root canal obturation and appeared to contribute to the significant reduction or complete regression of periapical lesions in teeth treated. The presented procedure may be proposed for everyday clinical practice.

Keywords: calcium hydroxide; chronic periapical lesions; dental injuries; MTA

INTRODUCTION

Traumatic tooth injuries are common in children and adolescents [1]. Large numbers of these injuries result in endodontic complications such as pulp inflammation, pulp necrosis, root resorption, obliteration of the root canal and development of periapical lesions [2]. Failure to comply with the recommendations of International Association of Dental Traumatology, with respect to clinical and radiographic follow-ups of injured teeth for a longer period of time, often results in a large number of unobserved endodontic complications, unless accompanied by subjective symptoms, like swelling or crown discoloration [3].

For decades, calcium hydroxide formulations have been a material of choice in the treatment of teeth with chronic periapical lesions because of their hygroscopic nature and strong antimicrobial activity [4]. In addition to their use as an inter-appointment intra-canal medicament, they have been proposed for pulp capping, pulp amputation, as well as in the treatment of root perforations, resorptive processes and fractured roots [4]. However, a possible shortcoming of the use of calcium-hydroxide product is the fact that its use lasts over relatively long period of time; also, there is a possibility of dentin weakening and susceptibility to root fracture [5]. Furthermore, it places a demand on the clinician to place a permanent type of restoration to prevent these possible adverse outcomes.

In the early 1990s, mineral trioxide aggregate (MTA) was presented as a material of choice for surgical root-end fillings [6]. Presently, MTA in all its variants and commercial products, is used in many endodontic and oral surgical procedures with considerable success [6, 7, 8]. MTA achieves good apical sealing, sets even in the presence of moisture and exhibits favorable biocompatibility and bioactivity [9, 10, 11]. Despite its use in various endodontic procedures and apparent advantages of the MTA when used as an apical plug, success in the management of traumatized teeth with chronic periapical lesions has minimal research support [8, 12].

The aim of this study was to assess the effectiveness of endodontic root canal procedures in traumatized permanent teeth with necrotic

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pulps and chronic periapical lesions, using tri-antibiotic paste and definitive obturation with MTA products. Healing was assessed by using the Photoshop CS (Adobe, San Jose, CA, USA) image-analysis software.

METHODS

Patients

The study involved 24 patients with a history of tooth trauma, 11 males and 13 females (mean age 13.30 ± 2.83), who came to the University Clinic, between January 2011 and July 2013, due to spontaneous or provoked pain, discomfort during chewing, numbness, or observed swelling. Based on the patients' subjective symptoms, clinical examination, vitality testing and analysis of periapical radiographs, acute exacerbation of necrotic pulp with chronic apical periodontitis was diagnosed, and the need for root canal treatment was determined.

Initially, during the patient screening process, periapical surgical treatment was recommended for all of the patients by their general dentist, and an oral surgery specialist also confirmed this recommendation. Upon personal request by the patients for a second opinion, an additional examination was conducted at the University Clinic.

Inclusion criteria for this study were healthy patients with non-vital tooth with chronic periapical lesions without root resorption, horizontal or vertical root fractures; exclusion criteria were unrestorable tooth, horizontal or vertical root fractures, and root resorption.

Attending parents were provided with a thorough written explanation of proposed non-surgical root canal procedures, their limitations, possible complications, length of the treatment, and observation period. Prior to the patients' participation in this study, written consent was obtained from the parents. This study was approved by the Ethics Committee and registered at the website www.clinicaltrials.org (NCT02625298).

Root canal treatment

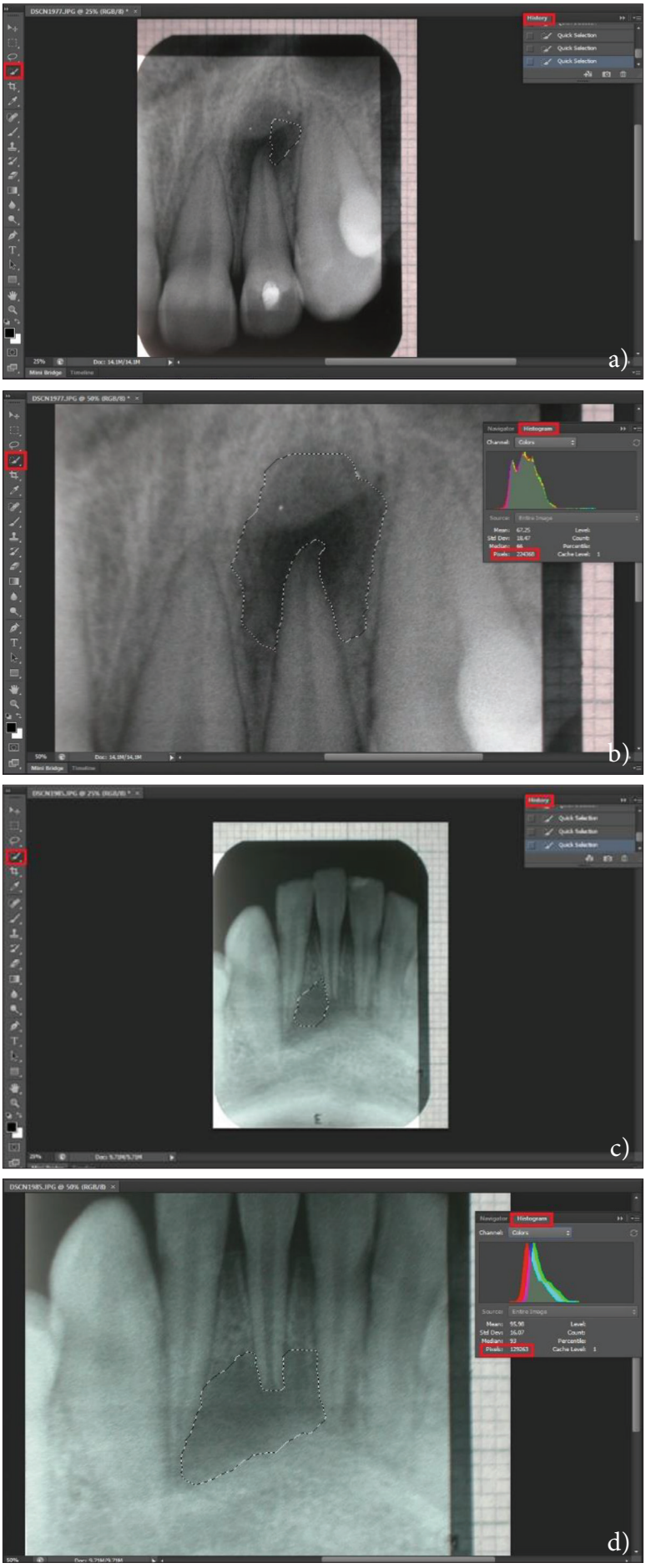
Root canal procedures were performed on 27 single-rooted traumatized permanent teeth (18 with mature and nine with immature root development). Following access opening in each tooth, the root canals were gently debrided with a crown-down manual technique using K-files (Dentsply, Maillefer, Switzerland) according to the radiographically determined working lengths. K-files were only used to remove the necrotic tissue and the softened predentinal layer without excessive removal of mineralized dentin, as this might further weaken already thin walls of the root canals, especially those with immature apical development. Subsequently, reshaping of the canal system, followed by a minor curettage of the periapical area through the canal using barbed broaches, was made in order to partially destruct the periapical lesion and provoke bleeding. Irrigation was performed using 2% NaOCl (Chloraxid, Cerkamed, Stalowa Wola, Poland), 0.2% solution of chlorhexidine-digluconate (Curasept 220, Curadent Swiss GmbH, Kriens, Switzerland) and 40% citric acid solution. The canals were then dried with sterile paper points and filled with calcium hydroxide paste (UltraCal XS, Ultradent Products Inc., South Jordan, UT, USA), which was left in the root canals for at least four weeks, with the maximum of six weeks (UltraCal XS, Ultradent Products Inc., South Jordan, UT, USA). Following this initial disinfection, triple-antibiotics paste (consisting of 200 mg of ciprofloxacin, 500 mg of metronidazole, and 100 mg of minocycline, with macrogol ointment and propylene glycol as carriers) was placed into the canal using a lentula, for a period of seven days. Subsequently, the apical thirds of the root canals were obturated either with ProRoot MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) or MTA^{+} (Cerkamed) by forming an apical plug 3–5 mm thick. The correct placement of the apical plug was assessed radiographically, and the moist cotton pellet was left in the root canal. The following day, the remaining canal space was filled with a sealer (Acroseal, Septodont, Saint-Maur des Frosses, France) and gutta-percha points (Guttapercha, VDW GmbH, Munich, Germany) using a lateral compaction technique. Coronal parts of root canal systems were sealed using glass-ionomer cement (Fuji IX, GC Int., Tokyo, Japan) with a minimum thickness of 1.5–2 mm. Enamel and dentin conditioning was performed with a self-etching adhesive system (GC G-BOND, GC Int.) and restored using a composite material (Gradia Direct, GC Int.) placed incrementally.

Clinical evaluation

Clinical evaluation of the performed endodontic treatment was done according to data obtained from patients' histories and clinical examinations. The positive clinical outcome comprised the absence of spontaneous or provoked pain, chewing without discomfort, absence of numbness or tenderness to percussion and/or palpation, and absence of tooth mobility, tooth crown discoloration or abscess, and/or sinus tract formation.

Radiographic analysis

Radiological assessment of the outcomes was performed according to the analysis of post treatment radiographs. Periapical radiographs, used for the initial assessment and diagnosis, were defined as the initial radiographs. Further progress in radiographic examination followed the procedures and requirements of the procedures rendered; the post treatment radiographs, made after definitive root canal obturation, were defined as the baseline radiograph (0 m), while the following control radiographs were secured at three, six, 12, and 24 months subsequent to obturation. Uniformity in radiographic exposures was provided with a silicone stabilizer for the purpose of positioning the X-ray tube. All radiographic images were taken using a periapical film (Kodak, Carestream Health Inc., Rochester, NY, USA) with a GE 1000 unit (General Electric, Milwaukee, WI, USA) at 90 kVp, 10 mA, and 0.12 seconds exposure time. The exposed films were developed



in an automatic processor (Dent-X 9000, Dent-X Co., Elmsford, NY, USA) using five minutes of dry-to-dry time.

Radiographs were photographed using a digital camera Kodak EasyShare Max (Z990) with a millimeter measurer in order to obtain the interpretation of sizes of periapical lesions during the conversion of pixels to mm^2 by digital data processing in Adobe Photoshop CS 6 software. Before the radiological assessment analysis, the brightness of the images was enhanced to facilitate observation of the periapical radiolucency. Using a histogram scale, the number of pixels for each lesion was noted (Figure 1 a–d). As the surface area of the image was known, and therefore the number of its pixels was also known, using the proportion, the size of the lesion was measured in mm^2. Criteria for radiographic assessment are presented in Table 1.

Table 1. Criteria for radiographic assessment

Success	· Decrease in size of the periapical lesion as compared with the previous radiograph · Measured value of the periapical lesion less than 3 mm^2 at the recall time of 24 months · No evidence of continuing root resorption · No evidence of root fracture
Uncertain outcome	· The size of periapical lesion remained the same
Failure	· Evidence that an existing periapical lesion has increased in size · Signs of continuing root resorption · Evidence of root fracture

Randomization and statistical analysis

Patients and clinicians were blinded to the treatment protocol (double-blinded randomization). One examiner (B.C.) randomized the entire sample using odd numbers. Two experienced and calibrated dentists (D.M. or T.P.) performed the radiological analysis independently. Inter-examiner and intra-examiner agreement scores were determined using the kappa

Figure 1. Analysis of radiographs using Adobe Photoshop CS 6 software:
a) marking the clear boundaries of periapical lesion step by step using the Quick Selection button;
b) determining the pixels of the marked periapical lesion using the Histogram;
c) marking clear boundaries of the periapical lesion step by step using the Quick Selection button;
d) determining the pixels of the marked periapical lesion using the Histogram

statistics. Data analysis was performed using the linear mixed model. The level of significance was set at $p < 0.05$, and the data was processed using IBM SPSS Statistics, version 20.0 (IBM Corp. Armonk, NY, USA).

RESULTS

Analysis of the patients' data history showed that none of the participants attended all of the control examinations designated by dental trauma protocols. Following an injury, 19 (79.2%) patients attended the first control examination, while after the third, sixth, and 12th month, this number decreased to 13 (54.2%), 10 (41.7%), and five (20.8%), respectively.

Mean time from the incidence of trauma to the occurrence of endodontic complication was 14.01 ± 2.69 months. A total sample consisted of three (11.1%) mandibular central incisors, 16 (59.3%) maxillary central incisors, seven (25.9%) maxillary lateral incisors and one mandibular first premolar (3.7%). Root canal procedures in this study lasted five to seven weeks (5.41 ± 0.67). The positive clinical outcome was recorded in 24 (88.9%) cases, while radiographic success was present in 26 (96.3%) cases (Tables 2 and 3). In one case (3.7%), due to the presence of a sinus tract and vertical root fracture, a single tooth was extracted, while two other cases (7.4%), with crown discolorations, were treated by carbamide peroxide as intracoronal bleaching agent.

The kappa statistics for intra-examiners' reliability ranged 0.69–0.89, while inter-examiners' reliability ranged 0.72–0.86. Statistically significant decreases in the values of the periapical lesions were recorded in all observation periods ($F = 115.966$, $p < 0.001$; Table 3). There were no statistically significant differences between the two used MTA materials ($F = 1.089$; $p = 0.306$), as well as between the teeth with mature and immature roots, regarding positive treatment outcomes ($p > 0.001$).

DISCUSSION

This study, in a certain sense, highlights the advantages of endodontic treatment in the initial management of traumatized teeth with chronic periapical periodontitis, as the initial suggestion was to perform periapical surgery in all the presented patients. On the other hand, results of the present study also show the importance of regular control follow-ups after tooth trauma, recommended by the relevant professional associations as control radiographs were made in two cases (8.3%) at the first control examination, after six months in three cases (12.5%), and no radiographs were made 12 months after the injuries [1, 3].

Trauma was identified as the etiological factor for pulp necrosis and chronic periapical lesions in all of the cases. The most predominant dental injuries were contusions in 13 (48.1%) cases, complicated crown fractures in five (18.5%) cases, while subluxations, uncomplicated crown fracture, as well as uncomplicated crown fractures in combination with teeth intrusions were present in three (11.1%) cases.

Management of teeth with necrotic pulps and chronic periapical lesions ranges from endodontic procedures and surgical approach to tooth extraction depending on the nature of the periapical lesion [13]. In this regard, an estimation of the volume of a periapical lesion is essential. Therefore, the main criterion for evaluation of the size of bone defects in the present study was the analysis of their 2D-radiograph reproductions as is common in daily practice. Using different tools in Adobe Photoshop CS software, the periapical radiolucency can be measured easily and with sufficient precision [14]. Ideally, cone-beam computed tomography evaluations might have provided a different outcome; however, their availability was not feasible at the time of this study, and also implies much higher effective dose of radiation (61–134 μSv) compared to conventional dental radiography (0.65–9.5 μSv) [14].

While there may be a significant correlation between the size of a periapical lesion and its true nature, the only

Table 2. Clinical status and outcome

Chewing discomfort (%)	Yes	No	48.1	51.9	7.4	92.6	0 100	0 100	0 100	0 100
Abscess/sinus tract (%)	Yes	No	48.1	51.9	0	100	0 100	3.7 96.3	0 100	0 100
Tooth discoloration (%)	Yes	No	-		-	-	0 100	0 100	3.7 96.3	3.7 96.3
Tooth mobility (%)	Yes	No	11.1	88.9	0	100	0 100	0 100	0 100	0 100
Numbness (%)	Yes	No	14.8	85.2	0	100	0 100	0 100	0 100	0 100
Percussion/ palpation tenderness (%)	Yes	No	92.6	7.3	7.4	92.6	0 100	0 100	0 100	0 100
Spontaneous/provoked pain (%)	Yes	No	37	63	0	100	0 100	0 100	0 100	0 100
Radiographic assessment intervals			Initial		Baseline		3 months	6 months	12 months	24 months

Table 3. Radiographic status and outcome

Time	Success	Uncertain outcome	Failure	Measured values of PL (mm^2) (MV±SD)
Initial	-	-	-	35.87 ± 0.24^{a}
Baseline	27 (100%)	0	0	$17.04 \pm 0.20^{a,b}$
3 months	26 (96.3%)	1 (3.7%)	0	$12.28 \pm 0.40^{b,c}$
6 months	26 (96.3%)	0	1 (3.7%)	$9.65 \pm 0.21^{c,d}$
12 months	26 (96.3%)	0	0	$6.52 \pm 0.17^{d,e}$
24 months	26 (96.3%)	0	0	0.31 ± 0.05^{e}

PL – periapical lesions, MV – mean value, SD – standard deviation;
^{a,b,c,d,e} statistically significant differences between measured values of PL ($p < 0.001$)

reliable proof of a correct diagnosis becomes possible after histopathological analysis [15, 16]. According to Nair [17], if the periapical lesion is completely separated from the apex of the tooth, it's less probably to be resolved without surgical treatment, but if it is in direct communication with the root canal, it may react favorably to a conservative form of management [18].

Integrity of the periapical area has been the subject of numerous studies in terms of both, instrumentation and medication. Bender [19] indicated that there are studies that claim that jeopardizing integrity of a periapical lesion may lead to an exacerbation of a chronic periapical process; however, Bhaskar [20] suggested that curettage of the periapical area may be useful in destruction or partial elimination of the affected tissue, and in initiating the reparatory processes. In the present study, this therapeutic procedure proved to be effective, though without any clear conclusions whether or not the outcome was due to the apical instrumentation, local pressure reduction, or provoked bleeding.

Numerous studies have shown that the use of calcium hydroxide in the treatment of periapical lesions is efficient, without significant differences between radiographically evaluated small (up to five mm) and large lesions [21, 22]. Although calcium hydroxide formulations possess powerful activity against a wide range of oral pathogens, they have limited effect against *E. faecalis* and *C. Albicans* [4]. As the use of the triple antibiotics may overcome the shortcomings of calcium hydroxide pastes, this was the main aim of its use in the present study, although for a shorter period of time than previously recommended [23, 24].

Annamalai and Mungara [25] reported complete absence of periapical radiolucency that was present at the begging of the treatment in 13/30 teeth with immature roots obturated with MTA. Using the periapical index score and the decrease in size of the apical lesion with at least 12 months follow-up, Simon et al. [26] demonstrated that the healing occurred in 81% of cases. Similar results were obtained by Holden et al. [27] and Sarris et al. [28]. The present results are in concordance with previous studies, which can also be explained by the fact that MTA-based materials possess a very similar chemical composition. Using MTA in this study as a root-end closure, both in teeth with compete and in those with incomplete root development, was undertaken because of the enhanced sealing ability of MTA, thereby reducing microleakage [6, 11], which is very important because the presence of residual microorganisms in dentinal tubules is considered the main reason for endodontic treatment failure.

Despite the broad scope of indications, there is still paucity of clinical studies on the use of MTA-based products *in vivo* when used in a manner similar to the present study. Furthermore, according to a literature search, there is a limited number of studies that followed the processes of repair and potential regeneration of chronic periapical lesions of traumatized permanent teeth obturated with MTA-based materials [29]. Even when the clinical situation indicates that a surgical intervention is necessary, continuous reduction of a periapical lesion over time following non-surgical intervention is a beneficial outcome. Moreover, other less invasive and successful options exist when necessary, like decompression of large periapical lesions [30].

CONCLUSION

Treatment of teeth with chronic periapical lesions, as a result of a complication of the previous tooth trauma, should be initiated with endodontic approach. Products based on MTA represent effective agents for apical root canal obturation and contribute to significant reduction or complete regression of periapical lesions. The presented procedure may be proposed for everyday clinical practice as it is easy to perform.

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Conflict of interest: None declared.

REFERENCES

1. Diangelis AJ, Andreasen JO, Ebeleseder KA, Kenny DJ, Trope M, Sigurdsson A, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxate on of permanent teeth. Dent Traumatol. 2012; 28(1):2–12.
2. Glendor U, Halling A, Andersson L, Eilert-Petersson E. Incidence of traumatic tooth injuries in children and adolescents in the county of Vastmanland. Sweden. Swed Dent J. 1996; 20(1-2):15–28.
3. Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, Diangelis AJ, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol. 2012; 28(2):88–96.
4. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J. 2011; 44(8):697–730.
5. Andreasen JO, Ban Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol. 2002; 18(3):134–7.
6. Srinivasan V, Waterhouse P, Whitworth J. Mineral trioxide aggregate in paediatric dentistry. Int J Paediatr Dent. 2009; 19(1):34–47.
7. Kottoor J, Velmurugan N. Revascularization for a necrotic immature permanent lateral incisor: a case report and literature review. Int J Paediatr Dent. 2013; 23(4):310–6.
8. Cetenovic B, Markovic D, Petrovic B, Peric T, Jokanovic V. Use of mineral trioxide aggregate in the treatment of traumatized teeth in children–Two case reports. Vojnosanit Pregl. 2013; 70(8):781–4.
9. da Silva GN, Braz MG, de Camargo EA, Salvadori DM, Ribeiro DA. Genotoxicity in primary human peripheral lymphocytes after exposure to regular and white mineral trioxide aggregate. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 102(5):e50–4.

10. Guven G, Cehreli ZC, Ural A, Serdar MA, Basak F. Effect of mineral trioxide aggregate cements on transforming growth factor beta1 and bone morphogenic protein production by human fibroblasts in vitro. J Endod. 2007; 33(4):447–50.
11. Chng HK, Islam I, Yap AU, Tong YW, Koh ET. Properties of a new root-end filling material. J Endod. 2005; 31(9):665–8.
12. Cohn SA. Treatment choices for negative outcomes with non-surgical root canal treatment: non-surgical retreatment vs. surgical retreatment vs. implants. Endod Topics. 2005; 11(1):4–24.
13. Van der Borden WG, Wang X, Wu MK, Shemesh H. Area and 3-dimensional volumetric changes of periapical lesions after root canal treatments. J Endod. 2013; 39(10):1245–9.
14. Carvalho FB, Gonçalves M, Tanomaru-Filho M. Evaluation of chronic periapical lesions by digital subtraction radiography by using Adobe Photoshop CS: a technical report. J Endod. 2007; 33(4):493–7.
15. Zain RB, Roswati N, Ismail K. Radiographic evaluation of lesion sizes of histologically diagnosed periapical cysts and granulomas. Ann Dent. 1989; 48(2):3–5.
16. Becconsall-Ryan K, Tong D, Love RM. Radiolucency inflammatory jaw lesions: a twenty-year analysis. Int Endod J. 2010; 43(10):859–65.
17. Nair PN. New perspectives on radicular cysts: do they heal? Int Endod J. 1998; 31(3):155–60.
18. Simon JHS. Incidence of periapical cysts in relation to the root canal. J Endod. 1980; 6(11):845–8.
19. Bender IB. A commentary on General Bhaskar's hypothesis. Oral Surg Oral Med Oral Pathol. 1972; 34(3):469–76.
20. Bhaskar SN. Nonsurgical resolution of radicular cysts. Oral Surg Oral Med Oral Pathol. 1972; 34(3):458–68.
21. Lin LM, Ricucci D, Lin J, Rosenberg PA. Nonsurgical root canal therapy of large cyst-like inflammatory periapical lesions and inflammatory apical cysts. J Endod. 2009; 35(5):607–15.
22. Maalouf EM, Gutmann JL. Biological perspectives on the non-surgical endodontic management of periradicular pathosis. Int Endod J. 1994; 27(3):154–62.
23. Trope M. Treatment of immature teeth with non-vital pulps and apical periodontitis. Endod Topics. 2006; 14(1):51–9.
24. Windley W 3rd, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod. 2005; 31(6):439–43.
25. Annamalai S, Mungara J. Efficacy of Mineral Trioxide Aggregate as an Apical Plug in Non-Vital Young Permanent Teeth: Preliminary Results. J Clin Pediatr Dent. 2010; 35(2):149–55.
26. Simon S, Rilliard F, Berdal A, Machtou P. The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study. Int Endod J. 2007; 40(3):186–97.
27. Holden DT, Schwartz SA, Kirkpatrick TC, Schindler WG. Clinical outcomes of artificial root-end barriers with mineral trioxide aggregate in teeth with immature apices. J Endod. 2008; 34(7):812–7.
28. Sarris S, Tahmassebi JF, Duggal MS, Cross IA. A clinical evaluation of mineral trioxide aggregate for root-end closure of non-vital immature permanent incisors in children-a pilot study. Dent Traumatol. 2008; 24(1):79–85.
29. Witherspoon DE, Small JC, Regan JD, Nunn M. Retrospective analysis of open apex teeth obturated with mineral trioxide aggregate. J Endod. 2008; 34(10):1171–6.
30. Gutmann JL, Ferreyra S. Alternative and contemporary management of large periradicular lesions. ENDO (Lond Engl). 2010; 4(2):127–44.

Ендодонтско лечење трауматизованих зуба са хроничним периапикалним лезијама применом антибиотске пасте и оптурације минералним триоксидним агрегатом – прелиминарна студија

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САЖЕТАК

Увод/Циљ Сврха ове студије била је процена ефикасности ендодонтског третмана трауматизованих сталних зуба са некротичном пулпом и хроничним периапикалним лезијама после дефинитивне оптурације минералним триоксидним агрегатом (MTA). Софтвер за анализу слике *Adobe Photoshop CS* коришћен је за процену регенерације.

Методе Двадесет и седам трауматизованих једнокорених сталних зуба са некротичном пулпом и хроничним периапикалним лезијама третирани су нехируршким процедурама помоћу триантибиотске пасте и калцијум-хидроксида као интраканалног медикамента. Дефинитивна оптурација изведена је помоћу *ProRoot* MTA или MTA$^+$ *Cerkamed*. Контролни прегледи обављени су три, шест, 12 и 24 месеца после завршетка лечења.

Резултати Позитиван клинички исход забележен је у 24 (88,9%) случаја, док је радиографски успех био присутан у 26 (96,3%) случаја. Статистички значајно смањење величине периапикалних лезија примећено је у свим периодима ($p < 0,001$). Није било статистички значајне разлике између два тестирана MTA материјала ($p > 0,05$).

Закључак MTA производи су ефикасни у оптурацији коренских канала и чини се да доприносе значајном смањењу или потпуној регресији периапикалних лезија у третираним зубима. Приказана методологија може бити предложена за свакодневну клиничку праксу.

Кључне речи: калцијум-хидроксид; хроничне периапикалне лезије; повреде зуба; MTA

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of symptoms and sings of oral soft tissue disorders among inpatients with schizophrenia

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SUMMARY

Introduction Patients with schizophrenia are likely to constitute a high-risk group of individuals with respect to prevalence of oral diseases and they require special attention. Factors like nature of psychiatric disorders, length of stay and oral-side effects of psychotropic medications have been noted as contributors to poor oral health among institutionalized chronic psychiatric patients.

Methods This cross-sectional study comprised 190 inpatients with schizophrenia at the Dr Laza Lazarevic Clinic for Mental Disorders in Belgrade, and 190 mentally healthy patients at the Clinic for Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade. A questionnaire was designed for the purpose of this research with the aim of recording information on demographic data (age and sex), unhealthy habits (tobacco smoking, alcohol consumption and drug abuse), and data about the existence of any oral symptom and/or sings related to oral soft tissue pathology. All participants were subjected to targeted clinical examinations.

Results All study group patients were receiving psychotropic medications (mean number 4.18 ± 1.14; from 1 to 7 medications). The study group patients had a total of 272 symptoms and 121 signs of oral disorders; which was almost four times higher for symptoms and even nine times higher for signs of oral disorders than in the control group.

Conclusion Schizophrenia as a mental disorder does not directly affect the condition of oral health of this group of psychiatric patients, but indirectly – reducing their motivation and awareness of the importance of oral health, which is particularly emphasized in hospital conditions.

Keywords: oral symptoms; oral signs; schizophrenia; hospitalization

INTRODUCTION

People with mental disorders are a part of the community deserving special attention. This group is often neglected by dental professionals due to ignorance, fear, stigma, misconceptions and negative attitudes. However, they are of even more concern because there is a loss of productivity due to their disability and an increased health care cost and burden to the government and society [1]. The majority of them who required long-term psychiatric care worldwide have schizophrenia diagnosed as their primary mental disorder [2]. The prevalence of schizophrenia is less than 1% in general population, without sex differences [3]. Treatment of institutionalized residents, especially those with schizophrenia, takes up an important part of the health care resource, compared to other psychiatric inpatients [4].

Having in mind oral health, hospitalized psychiatric patients are likely to constitute a high-risk group of individuals with respect to prevalence of oral diseases and they require special attention [5]. Factors like nature of psychiatric disorders, and oral-side effects of antipsychotic medications have been noted as contributors to poor oral health among institutionalized patients with schizophrenia [6]. In addition, unhealthy behaviors such as smoking cigarettes, alcohol consumption and drug abuse have been linked to psychiatric disorders [7].

Some studies that have been done on patients with schizophrenia focused on the assessment of dental caries and periodontal disease [8, 9, 10]. On the other hand, no published studies have addressed the prevalence of oral symptoms and disorders among inpatients with schizophrenia, or the influence of mental disorders on these conditions, although some studies recorded a high prevalence of oral symptoms and disorders, such as xerostomia, hypersalivation, recurrent oral ulcerations (RAS), burning mouth syndrome (BMS), tongue and lips disorders, oral lichen planus (OLP) etc. in psychiatric patients [11–14]. Therefore, the aim of this study was to assess the prevalence of symptoms and signs of oral disorders among inpatients with schizophrenia and to evaluate association of demographic, medical characteristics and unhealthy behaviors in this group of psychiatric inpatients with the development of oral soft tissue pathology.

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METHODS

This study was conducted as an observational cross-sectional study, in accordance with the Declaration of Helsinki and it was approved by the Ethics Committee of the Dr Laza Lazarevic Clinic for Mental Disorders in Belgrade, Serbia (No. 7221), and the Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia (No. 36/10). The study is reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for improving the quality of observational studies [15].

Each subject participated voluntarily in the study and was informed, through a special brochure regarding the type of research, data collection procedure, and other aspects of the study. Written consent was obtained from all subjects or their legal representatives to use personal data for research purposes. The study enrolled two groups of patients. The study group compromised 190 randomly selected patients with schizophrenia, hospitalized at the Dr. Laza Lazarevic Clinic for Mental Disorders in Belgrade, Serbia. The inclusion criteria for entering the study were that the patient was hospitalized, older than 18 years and diagnosed with schizophrenia (according to the 10. Revision of the International Classification of Diseases) two years prior to the study. Medical data for the study group patients (duration of psychiatric disease, number of hospitalizations, number and type of psychotropic medications) were taken from medical records. The exclusion criteria were a primary diagnosis of other mental disorder, hospitalized patients diagnosed with schizophrenia in the period shorter than two years from the time of the survey, the simultaneous presence of systematic diseases (e.g. nutritional deficiency, cardiovascular, respiratory, metabolic, endocrinal disorders), medications for such systematic diseases, and inability to communicate or the refusal to cooperate. The control group also comprised 190 healthy subjects suffering from aggressive periodontitis, without any psychiatric or somatic illness, who were consecutively recruited from the pool of patients at the Clinic for Periodontology and Oral Medicine, Faculty of Dental Medicine, University in Belgrade, Serbia [16]. Participants of the control did not use any medications that could affect oral health [17]. Groups were age- and sex- matched.

A questionnaire was designed for the purpose of this research with the aim of recording information on demographic data (age and sex), unhealthy habits (tobacco smoking, alcohol consumption and drug abuse), and data about the existence of any oral symptom and/or sings related to oral soft tissue pathology.

All participants were subjected to targeted clinical examinations in the dental office at the Dr Laza Lazarevic Clinic for Mental Disorders in Belgrade, Serbia (patients of the study group), and the Department of Periodontology and Oral Medicine, Faculty of Dental Medicine, University in Belgrade, Serbia (patients of the control group), according to criteria recommended by the World Health Organization [18]. The examination was performed in the following sequence: labial mucosa and labial sulci (upper and lower), labial part of the commissures and buccal mucosa (right and left), tongue (dorsal and ventral surfaces, margins), floor of the mouth, hard and soft palate and alveolar ridges/gingiva (upper and lower). During clinical examination, the following elements of the lesion were analyzed: anatomical location, extension; possible etiological or related factors were also recorded [19].

All collected data were organized and evaluated using dedicated software (SPSS 17.0 Inc., Chicago, IL, USA) and were analyzed by descriptive statistical parameters, methods for testing the difference of numerical data and regression models. Descriptive statistical methods were represented by measures of central tendency (mean and median), measures of variability (standard deviation and variation interval) and were expressed in percentages. The methods for testing the difference of numerical data (age) were represented by the t-test of independent groups. For testing data of different categories (sex, medications, unhealthy habits), χ^2 test was used. Level of significance was set at $p < 0.05$.

RESULTS

The study group consisted of 190 hospitalized patients with schizophrenia (95 males and 95 females) aged 19–67 years, with mean age of 43.59 ± 11.96 years. Most respondents (32.1%) were in age group over 50. The control group also consisted of 190 mentally healthy subjects (95 males and 95 females) aged 19–72 years, with mean age 43.20 ± 11.89 years. Most respondents in control group (30%) were in age group between 41–50. The groups thus where comparable in terms of age ($p = 0.747$ for t-test of independent groups) and sex ($p = 1.000$ for Pearson χ^2-test).

Distributions of unhealthy habits in both groups are shown in Table 1. In the study group most of the patients pleaded that they consume alcoholic beverages, in contrast to the control group who have often declared not to consume alcoholic beverages. Also, most of study group patients said that they sometimes enjoy drugs, unlike mentally healthy individuals who, in almost all cases, stated that they do not enjoy them. Among the patients of the study group, almost 75% of them smoked cigarettes; unlikely, in the control group patients there was less than half smokers. A statistically significant difference between the two groups of participants was observed in all three observed variables in terms of practicing bad habits for oral health (Table 1).

Table 1. Distribution of unhealthy habits in both groups

Unhealthy habits	Obtained values		Significance (p)[a]
	Study group n (%)	Control group n (%)	
Smoking cigarettes:			
yes	142 (74.7)	75 (39.5)	
no	48 (25.3)	115 (60.5)	[a]0.000*
Alcohol consumption:			
yes	49 (25.8)	2 (1.1)	
no	141 (74.2)	188 (98.9)	[a]0.000*
Drug abuse:			
yes	133 (70)	39 (20.5)	
no	57 (30)	151 (79.5)	[a]0.000*

*statistically significant;
[a]Pearson χ^2-test

Table 2. Psychotropic medications of patients in study group

Psychotropic medications	Obtained values Study group n (%)
Antipsychotics	
1) Typical antipsychotics	34 (17.9)
- chlorpromazine	8 (4.2)
- levopromazine	37 (19.5)
- fluphenazine	81 (42.6)
- haloperidole	
2) Atypical antipsychotics	
- clozapine	50 (26.3)
- risperidone	39 (20.5)
- quetiapine	4 (2.1)
- olanzapine	54 (28.4)
- sulpirid	2 (1.1)
- aripiprazole	3 (1.6)
Mood stabilizers	135 (71.1)
Hypnotics	63 (33.2)
Anxyolitics	160 (84.2)
Antidepressants	15 (7.9)
Antiparkinsonics	110 (57.9)

Table 3. Distribution of soft tissue pathology in both groups

Oral symptoms and signs	Obtained values		p[a]
	Study group n (%)	Control group n (%)	
Presence of oral symptoms:			
Burning mouth syndrome	122 (67.4)	50 (26.4)	0.000*
Facial pain	44 (24.3)	9 (4.7)	0.000*
Hypersalivation	22 (12.2)	0 (0)	0.000*
Xerostomia	19 (10.5)	2 (1.1)	0.000*
Halitosis	85 (47)	13 (6.8)	0.000*
Gustatory sense	50 (27.6)	36 (18.9)	0.048*
dysfunction	28 (15.5)	5 (2.6)	0.000*
Swallowing difficulties	24 (13.3)	0 (0)	0.000*
Presence of oral signs:			
Lips disorders	101 (55.8)	20 (10.6)	0.000*
Tongue disorders	23 (12.7)	2 (1.1)	0.000*
Soft and hard palate	47 (26)	4 (2.1)	0.000*
disorders	9 (5)	2 (1.1)	0.026*
Oral mucosa disorders	42 (23.2)	5 (2.6)	0.000*

*Statistically significant;
[a]Pearson χ^2 test

In the study group, schizophrenia lasted 14.31 ± 9.19 years on average (2–45 years). Most of patients (43.2%) have schizophrenia 11–20 years. The average number of hospitalizations per participant was 8.48 ± 5.71 (from one to 30). Sixty-eight patients with schizophrenia had less than ten hospitalizations, during the disease.

All study group patients were receiving psychotropic medications (mean number 4.18 ± 1.14; 1–7 medications) and in the greatest number – antipsychotics (mean number 1.64 ± 0.66; 1–3) (Table 2). In addition to antipsychotic medications, patients of the study group received other medications, too (Table 2). Almost 71% of inpatients with schizophrenia received mood stabilizers, 84.2% of them anxiolytics, 33.2% hypnotics, 7.9% antidepressants and 57.9% of them antiparkinsonics.

Based on the patients' subjective symptoms and clinical examination of the oral cavity, in both groups were noted some oral soft tissue diseases (Table 3). Almost half of the study group patients, and over 65% of the control group patients were free of any oral soft tissue pathology. The subjects of the study group had a total of 272 symptoms and 121 signs of oral disorders; which was almost four times higher for symptoms and even nine times higher for signs of oral disorders than in the control group. In the study group an average number of oral symptoms per patient were 1.26 ± 1.20 (range 0–5) and for signs of oral disorders 0.91 ± 1.21 (range 0–4), despite of respondents in the control group, where an average number of oral symptoms per patient were 0.29 ± 0.52 (range 0–2) and oral disorders 0.12 ± 0.35 (range 0–2). Distribution of the symptoms and signs of oral disorders in both groups of patients is shown in Table 3.

Univariate logistic regression showed that only on BMS statistical significance had duration of mental disorder and smoking cigarettes (Table 4). Similar to that, multivariate logistic regression showed a statistical significance of BMS among inpatients with schizophrenia in terms of mental disorder duration and smoking cigarettes (Table 4).

Table 4. Logistic regression of study group patients and oral soft tissue pathology

Observed characteristics	Obtained values significance (p)							
	Sex	Age	Duration of disease	Number of hospitalizations	Antipsychotics	Alcohol	Narcotics	Smoking
Burning mouth syndrome	[a]0.362	[a]0.620	[a]0.028*	[a]0.138	[a]0.655	[a]0.174	[a]0.180	[a]0.034*
	/	/	[b]0.012*	/	/	/	/	[b]0.005*
Facial pain	[a]0.034*	[a]0.904	[a]0.279	[a]0.486	[a]0.863	[a]0.876	[a]0.378	[a]0.730
Hypersalivation	[a]0.203	[a]0.670	[a]0.733	[a]0.290	[a]0.211	[a]0.519	[a]0.293	[a]0.829
Xerostomia	[a]0.405	[a]0.565	[a]0.983	[a]0.205	[a]0.308	[a]0.705	[a]0.321	[a]0.643
Halitosis	[a]0.129	[a]0.395	[a]0.963	[a]0.209	[a]0.895	[a]0.133	[a]0.254	[a]0.743
Gustatory sense dysfunction	[a]0.924	[a]0.692	[a]0.297	[a]0.523	[a]0.994	[a]0.502	[a]0.419	[a]0.568
Swallowing difficulties	[a]0.931	[a]0.406	[a]0.468	[a]0.988	[a]0.793	[a]0.539	[a]0.908	[a]0.353
Lips disorders	[a]0.890	[a]0.943	[a]0.959	[a]0.186	[a]0.641	[a]0.632	[a]0.129	[a]0.475
Tongue disorders	[a]0.008*	[a]0.518	[a]0.132	[a]0.340	[a]0.864	[a]0.928	[a]0.970	[a]0.463
Soft and hard palate disorders	[a]0.771	[a]0.465	[a]0.320	[a]0.141	[a]0.743	[a]0.227	[a]0.793	[a]0.521
Oral mucosa disorders	[a]0.293	[a]0.727	[a]0.874	[a]0.641	[a]0.217	[a]0.527	[a]0.758	[a]0.534

*Statistical significance;
[a]univariate logistic regression;
[b]multivariate logistic regression

DISCUSSION

Based on anamnesis' data and clinical examination of oral soft tissues, a statistically significant difference was found between inpatients with schizophrenia and mentally healthy patients in the presence of symptoms and signs of oral soft tissue diseases. Most commonly reported diseases were: xerostomia (43.2%), tongue illness (23.7%) and signs of buccal mucosa diseases (22.1%), as opposed to mentally healthy patients with the majority of cases registered with halitosis (18.9%). Xerostomia or "dry mouth" was the most common oral symptom that the inpatients with schizophrenia complained, which corresponds to the results of some previous studies [14, 19]. These results should not be surprising because it is known that xerostomia and hyposalivation may be the consequence of the application of some psychotropic medications including: first generation antipsychotics, antiparkinsonics, antidepressants, as well as anxiolytics, which are often applied to inpatients with schizophrenia in our study [19–23]. However, in a number of previous studies, xerostomia has been registered at significantly lower percentages of patients than in our research; Dangore-Khasbage et al. [24] registered xerostomia only in 13% of patients, Ujaoney et al. [25] in 22% of patients, while Morales-Chavez et al. [11] had xerostomia in only 9.23% of psychiatric patients. This can be explained by the fact that the researches concerned oral health of psychiatric patients (not only patients with schizophrenia), as well as that the patients in our study were treated with a greater number (1–3) of antipsychotics. Common habits such as smoking and alcohol consumption can cause some oral dryness [17]. The drugs most commonly implicated include antidepressants, antipsychotics, benzodiazepines, hypnotics, opioids, and substance abuse [17].

Most of inpatients with schizophrenia in this study had coated (n = 25) and black tongue (n = 12). Similar findings were also obtained in previous studies: Znegin et al. [14] found coated tongue in 8% of patients, while Bertaud-Gounot et al. [26] in 6.8% of patients. Coated tongue is a common oral-medical problem, due to accumulation of epithelial cells, residues of food and microbial debris [27]. It is well known that the coated tongue is occurring in a person with xerostomia and those who do not maintain or irregularly maintain oral hygiene [27]. Black tongue is a pathological change that also occurs in people who have poor oral hygiene, who smoke, have xerostomia, and consume soft and non-abrasive food [27]. In our research most of the inpatients with schizophrenia said they smoked (74.7%).

In addition, 22.1% examinees of the study group had some disease of oral mucosa, and the most common finding of RAS (n = 13) and OLP (n = 11) was consistent with the results of other investigators. Dangore-Khasbage et al. [24] reported RAS at 16% and OLP at 2% of patients; Bertaud-Gounot et al. [27] reported RAS in 12.4% of patients; Kossioni et al. [19] registered RAS in 3.6% of psychiatric patients, while Lai et al. [28] shown that olanzapine, quetiapine and sulpiride posed a higher risk of oral ulcerations among psychiatric patients, compared to the other antipsychotics. Also, it is known that RAS and OLP have psychosomatic support, highlighting the importance of anxiety, stress and depression in the development of these oral diseases. Cerqueira et al. [29] in their research indicate that psychological disorders (in particular anxiety and stress) have a high correlation with symptoms of OLP. Similar to that, Karthikeyan et al. [30] in their study indicate that stress can be a significant etiologic co-factor in OLP and RAS, which is interesting information that should be proven.

CONCLUSION

Based on the obtained risk factors for oral soft tissues diseases of schizophrenia inpatients, it can be said that schizophrenia as a mental disorder indirectly affect the condition of oral health of this group of psychiatric patients, by reducing their motivation and awareness of the importance of oral health, which is particularly emphasized in hospital conditions. Also, this research suggest that oral care of patients with schizophrenia must include periodic monitoring of dental and soft tissues, and that greater coordination between specialists of psychiatry and dentists may better serve the need of this neglect group of psychiatric patients.

Conflict of interest: None declared.

REFERENCES

1. Sangeeta UN, Rashmi S, Keshava PK. Periodontal health among non-hospitalized psychiatric patients in Mangaluru city – India. J Clin Diagn Res. 2016; 10(8):ZC40–3.
2. Chong HY, Teoh SL, Wu DB, Kotirum S, Chiou CF, Chaiyakunapruk N. Global economic burden of schizophrenia: a systematic review. Neuropsychiatr Dis Treat. 2016; 12:357–73.
3. Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, et al. Global epidemiology, and burden of schizophrenia: Findings from the global burden of disease study 2016. Schizophr Bull. 2018; 44(6):1195–203.
4. Harvey PD. What is so different about psychotic patients who have extremely long institutional stays? The American Journal of Geriatric Psychiatry. 2018; 26(2):198–9.
5. Yang M, Chen P, He MX, Lu M, Wang HM, Soares JC, et al. Poor oral health in patients with schizophrenia: A systematic review and meta-analysis. Schizophr Res. 2018; 201:3–9.
6. Grinshpoon A, Zusman SP, Weizman A, Ponizovsky AM. Dental health and the type of antipsychotic treatment in inpatients with schizophrenia. Isr J Psychiatry Relat Sci. 2015; 52(2):114–8.
7. Đorđević V. Đukić Dejanović S, Janković Lj, Todorović Lj. Shizophrenia and oral health – Review of the literature. Balkan Journal of Dental Medicine. 2016; 20(1):15–21.
8. Kisely S. No mental health without oral health. Can J Psychiatry. 2016; 61(5):277–82.
9. Wey MC, Loh S, Doss JG, Abu Bakar AK, Kisely S. The oral health of people with chronic schizophrenia: A neglected public health burden. Aust N Z J Psychiatry. 2016; 50(7):685–94.
10. Grinshpoon A, Zusman SP, Weizman A, Ponizovsky AM. Dental Health and the Type of Antipsychotic Treatment in Inpatients with Schizophrenia. Isr J Psychiatry Relat Sci. 2015; 52(2):114–8.

11. Morales-Chavez MC, Rueda-Delgado YM, Pena-Orozco DA. Prevalence of bucco-dental pathologies in patients with psychiatric disorders. J Clin Exp Dent. 2014; 6(1):e7–11.
12. Suresh KV, Shenai P, Charta L, Ronad YAA, Bilahari N, Pramod RC, et al. Oral mucosa diseases in anxiety and depression patients: Hospital based observational study from south India. J Clin Exp Dent. 2015; (1):e95–9.
13. Krunić J, Stojanović N, Ivković N, Stojić D. Salivary flow rate and decayed, missing, and filled teeth (DMFT) in female patients with schizophrenia on chlorpromazine therapy. Journal of Dental Science. 2013; 8(4):418–24.
14. Znegin AZ, Yanik K, Celenk P, Unal-Erzurumlu ZU, Yilmaz H, Bulut N. Oral hygiene and oral flora evaluation in psychiatric patients in nursing homes in Turkey. Niger J Clin Pract. 2015; 18(6):751–6.
15. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008; 61(4):344–9.
16. Benza-Bedoya R, Pareja-Vasquez M. Diagnosis and treatment of aggressive periodontitis. Odontoestomatologia. 2017; 19(30):29–39.
17. Scully C, Bagan JV. Adverse drug reactions in the orofacial region. Crit Rev Oral Biol Med. 2004; 15(4):221–9.
18. WHO. Oral health surveys – basic methods. World Health Organization, 2013.
19. Kossioni AM, Kossionis GE, Polychronopoulou A. Variation in oral health parameters between older people with and without mental disorders. Spec Care Dentist. 2013; 33(5):232–8.
20. Morio KA, Marshall TA, Qian F, Morgan TA. Comparing diet, oral hygiene and caries status of adult methamphetamine users and nonusers: a pilot study. J Am Dent Assoc. 2008; 139(2):171–6.
21. Thomas A, Lavrentzou E, Karouzos C, Kontis C. Factors which influence the oral condition of chronic schizophrenia patients. Spec Care in Dentistry. 1996; 16(2):84–6.
22. Hede B. Oral health in Danish hospitalized psychiatric patients. Community Dent Oral Epidemiol. 1995; 23(1):44–8.
23. Barnes GP, Allen EH, Parker WA, Lyon TC, Armentrout W, Cole JS. Dental treatment needs among hospitalized adult mental patients. Spec Care Dent. 1988; 8(4):173–7.
24. Dangore-Khasbage S, Khairkar PH, Degwekar SS, Bhowate RR, Bhake AS, Singh A, et al. Prevalence of oral mucosal disorders in institutionalized and non-institutionalized psychiatric patients: a study from AVBR hospital in central India. J Oral Sci. 2012; 54(1):85–91.
25. Ujaoney S, Motwani MB, Khairkar PH, Dewgekar SS, Bang G. Oral health status and dental treatment needs in institutionalized versus non-institutionalized psychiatric patients. Journal of Disability and Oral Health. 2010; 11(4):163–70.
26. Bernaud-Gounot V, Kovess-Masfety, Perrus C, Trohei G, Richard F. Oral health status and treatment needs among psychiatric inpatients in Rennes, France: a cross-sectional study. BMC Psychiatry. 2013; 13:227.
27. Janković Lj. Oralna medicina. Beograd: Zavod za udţbenike i nastavna sredstva; 2001.
28. Lai EC, Hsieh CY, Wong MB, Lin SJ, Yang YK, Yang YHK, et al. Comparative risk of oral ulcerations among antipsychotic users – population-based retrospective cohort study. Pharmacoepidemiol Drug Saf. 2015; 25(2):123–32.
29. Cerqueira JDM, Moura JR, Arsati F, De O. Lima-Asrati YB, Bittencourt RA, Freitas VS. Psychological disorders and oral lichen planus: A systematic review. J Investig Clin Dent. 2018; 9(4):e12363.
30. Karthikeyan P, Aswath N. Stress as an etiological co-factor in recurrent aphthous ulcers and oral lichen planus. J Oral Sci. 2016; 58(2):237–40.

Процена симптома и знакова оралних мекоткивних поремећаја код хоспитализованих болесника са схизофренијом

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САЖЕТАК

Увод Хоспитализовани болесници са схизофренијом могу да представљају високо ризичну групу особа са аспекта преваленције оралних обољења, те захтевају посебну пажњу. Фактори попут природе психијатријске болести, дужине хоспитализације и нежељених ефеката психотропних медикамената утичу на лоше орално здравље хоспитализованих хроничних психијатријских болесника.

Методе Ова студија пресека обухватила је 190 болесника са схизофренијом хоспитализованих у Клиници за психијатријске болести „Др Лаза Лазаревић“ у Београду и 190 ментално здравих болесника Клинике за парадонтологију и оралну медицину Стоматолошког факултета Универзитета у Београду. У сврху овог истраживања дизајниран је упитник са циљем бележења демографских података (старост и пол), лоших навика (пушење дувана, конзумирање алкохола и злоупотреба дрога) и података о постојању било којег оралног симптома и/или знака који се односи на патологију меких ткива усне дупље. Сви учесници били су подвргнути циљаним клиничким прегледима.

Резултати Сви болесници из студијске групе примали су психотропне лекове (средња вредност 4,18 ± 1,14, од једног до седам лекова). Болесници студијске групе имали су укупно 272 орална симптома и 121 знак оралних обољења, што је скоро четири пута више за оралне симптоме, а чак и девет пута више за знаке оралних поремећаја него у контролној групи.

Закључак Схизофренија као ментални поремећај не утиче директно на стање оралног здравља ове групе психијатријских болесника, већ индиректно – смањујући им мотивацију и свест о значају оралног здравља, посебно у болничким условима.

Кључне речи: орални симптоми; орални знаци; схизофренија; болничко лечење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Knowledge and attitudes on medical waste management among Belgrade medical and dental students

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SUMMARY

Introduction/Objective Knowledge and practical skills in medical waste (MW) management are of equal importance for medical and dental doctors. The first comparative study on the knowledge and skills in the field of MW management among Belgrade students was conducted with the goal of examining whether students of medicine and dentistry are equally familiar with this extremely important field.

Methods A cross-sectional study included 558 students of the sixth year of studies (430 medical and 128 dental students) who completed an anonymous semi-structured questionnaire to determine attitudes and knowledge on MW management.

Results The majority of medical and dental students had no training in MW management (79.5% and 74.6%, respectively). Dental students use protective equipment more frequently than medical students (94.5% *vs.* 42%, $p < 0.001$). However, full vaccinal protection against hepatitis B is better among medical students compared to dental students (57.7% *vs.* 39.1%, $p < 0.001$). Complete knowledge on post-exposal prophylaxis is better among medical students compared to dental students (44.5% *vs.* 13.3%, $p < 0.001$). However, dental students are more disciplined in reporting injuries (63.1% *vs.* 52.4%, $p = 0.038$). The students' knowledge on primary separation of infectious waste (93% *vs.* 77.8%, $p < 0.001$) and used needles (80.3% *vs.* 70.4%, $p = 0.007$) is better among dental students compared to medical students' correct answers.

Conclusion Dental students show better knowledge on MW management and are more disciplined in using personal infection protection compared to medical students. The students support continued training on MW management and investigations on this topic.

Keywords: medical waste; safety; education; medical students; dental students

INTRODUCTION

The term medical waste (MW) refers to all the waste generated within health-care facilities, research centers and laboratories. It consists of materials ranging from used needles to body parts, diagnostic samples, blood, chemicals, pharmaceuticals, and radioactive materials. From 10% to 25% of all MW is hazardous and may cause a variety of environmental and health risks [1, 2].

A quarter of all MW in Serbia is hazardous, and infectious MW is the largest part of it [3, 4]. Annual production of infectious waste in Serbia is between 4,500 and 5,000 tons [5]. Since 2006, the national system for safe MW management has been put into place and all infectious MW has been sterilized. Serbia has reduced the amount of hazardous MW by 50% by introducing a waste separation process in healthcare facilities [6].

Recognizing the importance of familiarity with MW management for healthcare professionals, Serbian medical faculties have recently introduced MW topic into the curriculum in the final year of their studies. To assess the quality of undergraduate education on MW management, it is important to check the students' retention of knowledge. Further, although the knowledge in this field is of equal importance for medical and dental doctors, it is not clear whether medical and dental students adopt this necessary knowledge equally. For these reasons, we undertake this comparative study on knowledge and attitudes on MW management among Belgrade medical and dental students.

METHODS

We undertook a cross-sectional study between December 2017 and January 2018 at the Faculties of Medicine and Dentistry, University of Belgrade, Serbia. The study comprised 558 students of the sixth year of studies, 430 medical students (response rate 92.47%) and 128 dental students (response rate 81.01%). There were

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more female respondents in both samples, 62.8% among medical and 64.1% among dental students.

We used an original semi-structured questionnaire designed for this study to determine knowledge, attitude, and practice concerning MW management. Students were recruited during their classes and participation was voluntary and anonymous. The questionnaire consisted of four parts. Some questions were taken from the questionnaires from similar studies but were not standardized, which was the case with ours as well.

The first part of the questionnaire included questions concerning training for MW management, wearing personal protection (mask, gloves, protective glasses), knowledge about post-exposal prophylaxis, and the vaccinal status of students (10 questions). The second part of the questionnaire comprised questions on MW regulation, management (segregation, internal collection, packaging, storage, and final disposal) and injury reporting system (41 questions). The third part of the questionnaire referred to the knowledge about color coding system (four questions). The fourth part of the questionnaire was in the form of five graded Likert's scale statements (1= "I fully disagree"; 2 = "I mainly disagree"; 3 = "I cannot decide"; 4 = "I mainly agree"; 5 = "I fully agree") concerning MW management, continuing training, and investigations on this topic (seven questions).

Statistical analysis

We performed statistical analysis with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). We set the significance level at 0.05. The distribution of categorical variables was investigated with χ^2 test. To test the significance of differences between the mean values of numeric and ordinal variables we used Student's t-test and Mann–Whitney U test, respectively.

Ethical consideration

We performed the study with the permission from the Ethics Committee of the Faculty of Medicine, University of Belgrade.

RESULTS

The distribution of medical and dental students was similar concerning the training in MW management ($\chi^2 = 1.516$; $p > 0.05$). The majority of students had no training (74.6% at the Faculty of Medicine and 79.5% at the Faculty of Dental Medicine); a small number of them had partial training (18.5% *vs.* 15.6%); those who had full training were very few (6.9% *vs.* 4.9%).

Dental students use protective equipment more frequently than medical students (Table 1). Male students are more disciplined in this regard compared to their female colleagues (56.9% *vs.* 53%; $\chi^2 = 6.446$; $p = 0.04$). However, vaccinal protection against hepatitis B is better among medical students than in dental students (Table 1).

The knowledge on post-exposal prophylaxis is better among medical students compared to dental students (44.5% *vs.* 13.3%, $\chi^2 = 66.308$; $p < 0.001$); the availability of post-exposal prophylaxis is also better at the medical faculty compared to the dental one (36.4% *vs.* 14.8%, $\chi^2 = 31.783$; $p < 0.001$).

Around 80% of students of both faculties are aware of the significance of reporting injuries at work. However, dental students are more disciplined in reporting injuries compared to medical students (63.1% *vs.* 52.4%, $\chi^2 = 4.318$; $p = 0.038$). The responses of students are similar in relation to the treatment of injuries from sharp objects.

The majority of students are not familiar with the legal regulations regarding MW management, nor with the latest provisions from 2016, but most of them know who the responsible person is for managing MW at their faculty. Concerning waste separation at the faculty, there are more dental than medical students who believe that their faculty separates waste (Table 2).

Table 1. Infection protection among Belgrade medical and dental students

Infection protection	Answer	Faculty			p*
		Medicine n (%)	Dentistry n (%)	Total n (%)	
Personal protective devices	No	101 (24.4)	3 (2.3)	104 (19.2)	<
	Yes, fully	174 (42)	121 (94.5)	295 (54.4)	
	Yes, partially	139 (33.6)	4 (3.1)	143 (26.4)	
	Totally	414 (100)	128 (100)	542 (100)	
Vaccination against hepatitis B	No	159 (37.1)	76 (59.4)	535 (42.3)	<
	Yes, fully	247 (57.7)	50 (39.1)	297 (53.4)	
	Yes, partially	22 (5.1)	2 (1.6)	24 (4.3)	
	Totally	428 (100)	128 (100)	556 (100)	

*χ^2 test

Table 2. Knowledge and attitudes on medical waste management among Belgrade medical and dental students

Knowledge and attitudes on medical waste management	Answer	Faculty		p*
		Medicine n (%)	Dentistry n (%)	
Legal regulation	No	394 (91.8)	111 (86)	0.049
	Yes	35 (8.2)	18 (14)	
Responsible person at the faculty	No	365 (90.3)	119 (93.7)	> 0.05
	Yes	39 (9.7)	8 (6.3)	
Waste separation at the faculty	No	164 (41.2)	22 (17.2)	< 0.001
	Yes	234 (58.8)	106 (82.8)	
Containers for medical waste at the faculty	No	55 (13)	20 (15.5)	> 0.05
	Yes	367 (87)	109 (84.5)	

Concerning the primary separation of MW, the students' knowledge on infectious waste and used needles was satisfactory and better among dental students compared to medical ones. However, the majority of students of both faculties gave incorrect answers related to chemical and pathoanatomic waste, heavy metals, and cytotoxic drugs (Table 3).

Dental students showed more positive attitude towards MW management compared to medical students, particularly concerning the continuation of training and investigations on this topic (Cronbach's alpha = 0.778) (Table 4).

Table 3. Knowledge on primary separation of medical waste among Belgrade medical and dental students

Primary separation of medical waste	Faculty				p*
	Medicine		Dentistry		
	Answer n (%)		Answer n (%)		
	Correct	Incorrect	Correct	Incorrect	
Infectious waste – yellow	330 (77.8)	94 (22.2)	120 (93)	9 (7)	< 0.001
Chemical waste – purple	146 (35.4)	266 (64.6)	43 (35.5)	78 (64.5)	> 0.05
Pathoanatomic waste – brown	179 (46.3)	208 (53.7)	50 (43.5)	65 (56.5)	> 0.05
Used needle – yellow	280 (70.4)	118 (29.6)	98 (80.3)	24 (19.7)	0.007
Thermometer with mercury – violet	26 (6.5)	372 (93.5)	12 (9.8)	110 (90.2)	0.012
Syringe / cytotoxic drugs – red	127 (32.7)	261 (67.3)	31 (26.5)	86 (73.5)	> 0.05

*χ^2 test

Table 4. Attitudes towards medical waste management among Belgrade medical and dental students (5-graded scale of agreement; mean ± standard deviation)

Attitude	Medical students	Dental students	p*
Inadequate medical waste management affects human health and environment	4.72 ± 0.69	4.71 ± 0.79	0.646
Medical waste management should be a mandatory part of practical and theoretical training of medical and dental students	4.01 ± 1.06	4.37 ± 0.92	< 0.001
Training on medical waste management should be performed at least once a year	3.71 ± 1.10	3.96 ± 1.08	0.020
In my opinion, medical waste is a topic that deserves more attention in the future	4.27 ± 0.89	4.49 ± 0.79	0.004
More investigations on medical waste management are needed	3.95 ± 1.12	4.24 ± 1.03	0.005

*Mann–Whitney U test

DISCUSSION

The majority of students of both faculties finish their studies with no training in the management of MW. Similar results were obtained in a study among health professional students in India, with only 19% of them trained in MW management [7]. Similarly, only about 40% of doctors employed in healthcare centers in Nigeria received adequate training on MW [8]. Another study conducted in Jahor showed that 37% of health workers did not pass adequate training in handling and disposal of sharp objects, with a significantly lower incidence of stabbing on sharp objects among those who were trained in MW compared to those who were not [9].

Dentistry students are disciplined in using protective equipment at work (94.5%); however, the majority of them have not been vaccinated against hepatitis B (59.4%). A minor part of dentistry students is familiar with post-exposal prophylaxis (13.3%), and half of them claim that it is not available. Unlike dental students, medical students are more aware of the significance of vaccination against hepatitis B; but, they use protective equipment in lesser degree (42%), and only a third are familiar with post-exposal prophylaxis. Still, the situation in Serbia in this regard is better than in Nigeria [8] and in Tanzania [10] where post-exposal prophylaxis is familiar to a lesser degree both to dental and medical students (30% and 22.5%, respectively). The relevant results are much better in Batu Pahat, Johor, Malaysia, where 87% of medical practitioners confirmed the use of personal protective equipment in handling clinical waste [9].

The vaccinal protection of health workers in Serbia is significantly better than in Nigeria, where only 18.5% of health workers are adequately vaccinated against hepatitis B [8]. Medical students are more aware of the significance of vaccination against HBV and are more frequently vaccinated compared to dental students (57.7% *vs.* 39.1%, respectively). Immunization against HBV is of utmost importance for all health workers [11]. There are countries that fully recognize this fact and have much better vaccination results. For instance, Saudi Arabia has 80% of dental students vaccinated against hepatitis B [12].

Undergraduate healthcare students undergo professional practice that exposes them to biological material. A study conducted in São Paulo, Brazil, registered that 48.8% of students' accidents with biological material occurred among dentistry students, 40.6% among medical students, and 6.5% among nursing students [13]. The practice of reporting injuries resulting from improper waste disposal is very poor in Serbia, in spite of the awareness of reporting significance, confirmed in our study among approximately 80% of students. In the present study we show that only one half of medical students and 63% of dentistry students report injuries from MW; still this is better than in India, where the practice of reporting injuries from MW is between 39.2% and 45.6% [8]. The practice of reporting work-related injuries caused by improper MW disposal is very poor across all groups of health professionals in India [14]. In developing countries, medical workers do not report about 40–75% of injuries from sharp objects; this is a major problem especially in case of HIV infection, where post-exposal prophylaxis is effective in 80% of the cases [15]. Even in developed countries like Poland, injury reporting in hospitals is low; the implementation of regulations in this field did not help [16].

According to the data of the Public Health Institute of Serbia, stab injuries from MW are poorly reported in Serbia; training of health workers may be an effective strategy for improving the practice and behavior towards hospital MW management [17]. In Serbia, 172 seminars were held 2008–2014; 3,278 employees in health care were trained

at three levels – technician, supervisor, and manager of waste management [6]. However, there was no adequate training of students.

A small percentage of Belgrade medical and dental students, 8.2% and 14%, respectively, are well informed about the regulations related to MW management. Dental students in India are better informed about MW management; 55.5% of them had good training and 31% of them even know the year when this law was established [18, 19]; in a study conducted in 2016, this number increased to 64.3% [20]. Similarly to our results, the majority of health workers in Brazil are not familiar with regulations related to MW [21].

Regardless of poor knowledge on regulations, 90% of the investigated students know the person responsible for MW; this is better than in Jahor, Malaysia, where 83% of the respondents know the answer [9]. A recent study showed that the presence of waste managers can effectively minimize the risk of infection [22].

Most of our respondents, especially among dentistry students, know how to dispose of infectious waste and sharp objects; this is similar to the results of a study among the medical staff in the Babol City Hospital, Iran; 97% of them knew MW color coding [23]. However, the majority of students in our study did not know the proper way of managing chemical (90%) or cytotoxic waste (70%). These results are in contrast to a study among dental students from India, where 67% of the participants demonstrated good knowledge about disposing pharmaceuticals [24]. Similar results were found in a study conducted in Cairo, in which 60.9% of doctors answered correctly regarding the disposal of chemical waste [25]. Better knowledge on the management of infectious waste compared to other hazardous MW is probably related to the dominance of this type of MW in hospitals.

A recent review of literature has indicated that in many developing countries regulations and laws relating to waste management in hospitals have been adopted recently. However, the knowledge and awareness of adequate MW management remain poor due to the lack of appropriate training for both doctors and hospital staff, although this is a necessity today [26, 27]. A study conducted among Brazilian dental students proved that theoretical knowledge on waste managements can be improved, but this may not amend waste segregation and adequate disposal in dental practice [28]. In our study, students show a very positive attitude towards continuing training on MW management and investigations on this topic.

CONCLUSION

In this very first study on the knowledge and awareness of MW among Belgrade medical and dental students, we show that their training in MW management and vaccinal protection against hepatitis B are unsatisfactory. Dental students show better knowledge on MW and are more disciplined in personal protection compared to medical students. The students support better training on MW management at their faculties and more investigations on this topic.

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REFERENCES

1. World Health Organization (WHO) Policy Paper: Safe Health Care Waste Management. Geneva, Switzerland: World Health Organization (WHO); 2014.
2. Health care waste. At: http://www.who.int/mediacentre/factsheets/fs253/en/ Accessed: February 22, 2018.
3. Milanović S, Jovanovic V, Mršulja A, Stojčić M, Ilić Živojinović J. Medical waste management. A handbook for students of high medical schools. Belgrade: Zavod za udžbenike, 2012 (in Serbian).
4. The Law on Waste Management of the Republic of Serbia. Official Gazette, No. 36/2009, 88/2010 and 14/2016
5. Jovanović V, Tesink JG, Jovanović D, Đonović N, Jevtić M, Matić B, et al. Development of Healthcare Waste Management in Serbia and Challenges in the Improvement of the Quality of Healthcare Services. Tehnika – Kvalitet, standardizacija i metrologija. 2014; 14(2):343–8.
6. Knezevic S, Matic B. A report on the follow-up process on infectious medical waste management within the network of state health facilities in the Republic of Serbia in 2013. Regulated waste flows. Centre for Hygiene and Human Ecology. Republic Institute of Public Health of Serbia (http://www.batut.org.rs/download/izvestaji/higijena/2013MedOtpad.pdf).
7. Kumar M, Kushwaha R, Kumari M, Singh G, Kumari R. Knowledge, awareness, attitude regarding biomedical waste management among medical students in a tertiary care centre: A cross sectional Study. PARIPEX Indian Journal of Research. 2017; 6(4):611–4.
8. Anozie OB, Lawani LO, Eze JN, Mamah EJ, Onoh RC, Ogah EO, et al. Knowledge, Attitude and Practice of Healthcare Managers to Medical Waste Management and Occupational Safety Practices: Findings from Southeast Nigeria. J Clin Diagn Res. 2017; 11(3):IC01–IC04.
9. Nazli SN, Karuppannan SA/L, Omar D. Knowledge and Awareness of Clinical Waste Management among Medical Practitioners in Hospital Batu Pahat, Johor. IJIMT. 2014; 5(2):139–42.
10. Mponela MJ, Oleribe OO, Abade A, Kwesigabo G. Post exposure prophylaxis following occupational exposure to HIV: a survey of health care workers in Mbeya, Tanzania, 2009-2010. Pan Afr Med J. 2015; 21:32.
11. Gupta S, Rani S, Garg S. Infection control knowledge and practice: A cross-sectional survey on dental laboratories in dental institutes of North India. J Indian Prosthodont Soc. 2017; 17(4):348–54.
12. Ahmad IA, Rehan EA, Pani SC. Compliance of Saudi dental students with infection control guidelines. Int Dent J. 2013; 63(4):196–201.
13. Gir E, Netto JC, Malaguti SE, Canini SRMS, Hayashida M, Machado AA. Accidents with biological material and immunization against hepatitis B among students from the health area. Rev Lat Am Enfermagem. 2008; 16(3):401–6.

14. Vanesh Mathur, S Dwivedi, MA Hassan, RP Misra. Knowledge, Attitude, and Practices about Biomedical Waste Management among Healthcare Personnel: A Cross-sectional Study. Indian J Community Med. 2011; 36(2):143–5.
15. Wilburn SQ. Needlestick and sharps injury prevention. Online J Issues Nurs. 2004; 9(3):5.
16. Garus-Pakowska A, Górajski M, Szatko F. Did legal regulations change the reporting frequency of sharp injuries of medical personnel? Study from 36 hospitals in Łódź Province, Poland. Int J Occup Med Environ Health. 2018; 31(1):37–46.
17. Kumar R, Somrongthong R, Ahmed J. Impact of waste management training intervention on knowledge, attitude and practices of teaching hospital workers in Pakistan. Pak J Med Sci. 2016; 32(3):705–10.
18. Ranjan R, Pathak R, Singh DK, Jalaluddin MD, Kore SA, Kore AR. Awareness about biomedical waste management and knowledge of effective recycling of dental materials among dental students. J Int Soc Prev Community Dent. 2016; 6(5):474–9.
19. Saini R, Pithon MM, Singh HK, Popoff DV. Knowledge of Biomedical Waste Management among the Students of Rural Dental College, Maharashtra, India. Int J Experiment Dent Sci. 2013; 2:24–6.
20. Singh LD, Elangbam V, Napolean T, Christina S. Knowledge on Biomedical Waste Management among Medical Students in RIMS, Imphal, Manipur. IOSR-JDMS. 2018; 17(01):40–3.
21. Assis MC, Gomes VAP, Balista WC, Freitas RR. Use of performance indicators to assess the solid waste management of health services. An Acad Bras Cien. 2017; 89(3):2445–60.
22. Maina SM, Andrew NK, Caroline WN. Assessment of Level of Knowledge in Medical Waste Management in Selected Hospitals in Kenya. Appli Micro Open Access. 2016; 2:124.
23. Amouei A, Fallah SH, Asgharnia HA, Gholami M, Jafarian S. Knowledge and Attitude of Hospital Personnel Regarding Medical Waste Management. Int Arch Health Sci. 2015; 2:75–81.
24. Sood AG, Sood A. Dental perspective on biomedical waste and mercury management: A knowledge, attitude and practice survey. Indian J Dent Res. 2011; 22(3):371–5.
25. Hakim HA, Mohsen A, Bakr I. Knowledge, attitudes and practices of health-care personnel towards waste disposal management at Ain Shams University Hospitals, Cairo. East Mediterr Health J. 2014; 20(5):347–54.
26. Ali M, Wang W, Chaudhry N, Geng Y. Hospital waste management in developing countries: A mini review. Waste Manag Res. 2017; 35(6):581–92.
27. Ozder A, Taker B, Eker HH, Altindis S, Kocaakman M, Karabay O. Medical waste management training for healthcare managers - a necessity? J Environ Health Sci Eng. 2013; 11(1):20.
28. Victorelli G, Flório FM, Ramacciato JC, Motta RHL, de Souza Fonseca Silva A. Impact of Pedagogical Method on Brazilian Dental Students' Waste Management Practice. J Dent Educ. 2014; 78(11):1528–33.

Знање и ставови београдских студената медицине и стоматологије о управљању медицинским отпадом

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САЖЕТАК

Увод/Циљ Знање и практичне вештине управљања медицинским отпадом су од посебног значаја за докторе медицине и стоматологије. Компаративна студија о знању и вештинама из области управљања медицинским отпадом спроведена је међу београдским студентима први пут, у циљу испитивања да ли овом изузетно важном облашћу студенти медицине и стоматологије владају подједнако добро.

Методе Ова студија пресека обухватила је 558 студената шесте године студија на Београдском универзитету (430 студената медицине и 128 студената стоматологије), који су попунили анонимни упитник о знању и ставовима о управљању медицинским отпадом.

Резултати Већина студената стоматологије и медицине нису имали никакву посебну обуку из управљања медицинским отпадом (79,5% и 74,6%). Студенти стоматологије су чешће користили заштитну опрему (94,5% према 42%, $p < 0,001$). Вакцинална заштита од хепатитиса Б је комплетнија међу студентима медицине у односу на студенте стоматологије (57,7% према 39,1%, $p < 0,001$). Знање о профилакси после изложености боља је међу студентима медицине (44,5% према 13,3%, $p < 0,001$). Међутим, студенти стоматологије су ажурнији у погледу пријављивања повреда на радном месту (63,1% према 52,4%, $p = 0,038$). Знање студената о примарној сепарацији инфективног отпада и коришћених игала је боље међу будућим стоматолозима (тачни одговори 93% насупрот 77,8%; $p < 0,001$ и 80,3% према 70,4%; $p = 0,007$).

Закључак Студенти стоматологије имају боље знање о управљању медицинским отпадом и дисциплинованији су у погледу коришћења заштитне опреме на раду у односу на студенте медицине. Студенти подржавају континуирану едукацију о управљању медицинским отпадом и даља истраживања о овој теми.

Кључне речи: медицински отпад; сигурност; едукација; студенти медицине; студенти стоматологије

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The synergistic action of antioxidative enzymes – correlations of catalase and superoxide dismutase in the development and during the treatment of type 2 diabetes

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SUMMARY

Introduction/Objective The wider literature review of analysis in levels of catalase (CAT) or superoxide dismutase (SOD) enzymes in type 2 diabetes mellitus (T2DM) patients shows no pronounced consistency. We have assumed that the onset of diabetes does not significantly change individual quantities of these enzymes, but instead it changes the relationship of these enzymes.

Methods The study consisted of four groups (n = 30 for each group): obese individuals with disturbed glucose metabolism (subjects with newly diagnosed T2DM) before and after metformin treatment initiation, obese subjects with normal glucose tolerance (NGT) and a control group of healthy normal weight subjects. Appropriate anthropometric measurements and laboratory tests of biochemical parameters and antioxidative enzymes were carried out in all participants.

Results Our study has confirmed that correlation of enzymes CAT and SOD is significantly changed in patients with newly diagnosed T2DM, and that it can be restored by reestablishment of glucose homeostasis with adequate antidiabetic treatment.

Conclusion The applied therapy restores the dynamic balance of CAT and SOD, mainly through the reintegration of the new equilibrium in the enzyme system after achieving better glycemic control. These conclusions are only valid in the initial stages of T2DM treatment.

Keywords: antioxidative enzymes; obesity; glucose metabolism; metformin

INTRODUCTION

Oxidative stress is one of the important factors contributing to the pathogenesis of the large number of diseases such as obesity, diabetes, atherosclerosis, inflammatory, malignant and certain neurodegenerative diseases [1, 2]. The enzyme superoxide dismutase (SOD) protects the cells from superoxide anion radicals entering into the chemical reaction and turning these radicals into hydrogen peroxide, which is further detoxified to H_2O in the lysosomes through the enzyme catalase (CAT), or in the mitochondria through the enzyme glutathione peroxidase (GPX) [3].

Hyperglycemia, increased intake of free fatty acids and excessive exposure to ultraviolet radiation are leading to increased oxidative stress, but the role of antioxidant enzymes is still not fully clarified [4, 5]. Previous studies came to conflicting results regarding the activities of these antioxidant enzymes in diabetic patients. Levels of SOD in diabetic patients were found to be significantly elevated, significantly reduced [6, 7], or unchanged in comparison to the control group [8]. Similarly, other authors concluded that CAT level is significantly higher, significantly lower, or is the same as in individuals from the control group [9, 10, 11].

For the purpose of clarifying the role of some of antioxidant enzymes, we studied the functional association between glycemia and CAT and SOD function. Although number of previous studies already assessed levels of CAT and SOD in type 2 diabetes (T2DM) patients, correlation of these two enzymes has rarely been the subject of previous researches. The principal aim of this study is to examine functional association of these two enzymes, which may be crucial for the optimal antioxidative protection achievement. Secondary, this study aims to evaluate the possible influence of the metformin therapy on these enzymes in diabetic patients.

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Table 1. Parameters and distributions (mean value and standard deviation) for SOD, CAT, fasting and postprandial 2 h glucose (G0H and G2H) in study groups

	SOD (U/g) (Figure 1)	CAT (U/mL) (Figure 2)	G0H (mmol/l) (Figure 3)	G2H (mmol/l)
Control group	N (303.28; 57.73)	N (-141.24; 47.13)	N (4.54; 0.35)	N (4.952; 0.654)
Obese	N (247,05; 55.25)	N (-147.35; 37.61)	N (5.06; 0.54)	N (5.528, 0.965)
T2DM group before the treatment	X (283.72; 122.35)	N (-140.62; 39.92)	X (9.48; 3.72)	X (12.74; 5.42)
T2DM group after the treatment	U (238.82; 35.91)	U (-128.95; 35.91)	lnN (7.42; 2.45)	lnN (9.307; 3.218)

Types of distribution (N – normal; U – uniform; lnN – lognormal; X – undefined); T2DM – type 2 diabetes mellitus; SOD – superoxide dismutase; CAT – catalase; G0H – fasting glucose; G2H – postprandial 2 h glucose

METHODS

The study was conducted at the Clinic for Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina, Novi Sad, Serbia and it enrolled 90 age- and sex-matched individuals who gave their written consent prior to participation in the study. The study was carried out in accordance with Helsinki declaration and it was approved by the local ethical committee.

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m) (kg/m^2). We excluded individuals with all chronic conditions that affect the oxidative status of the organism.

Subjects were divided in the following groups:

1) Thirty obese individuals with normal glucose tolerance and normal distribution of BMI (37.37 ± 6.11 kg/m^2);

2) Thirty obese individuals with newly diagnosed T2DM and normal distribution of BMI on the baseline (34.41 ± 4.68 kg/m^2) analyzed before and during metformin therapy;

3) Thirty healthy normal weight control individuals with a normal distribution of BMI (23.34 ± 3.12 kg/m^2).

The values of glycemia, CAT and SOD in Group 2 were analyzed before and three months after the initiation of metformin therapy (1,000 mg per day in all subjects).

Blood was sampled for the analysis of various parameters. Fasting and two-hour postprandial glycose were determined by enzymatic methods. Determination of parameters of oxidative stress was performed after the following preparation of the blood sample: 0.5 mL of heparinized blood was centrifuged for 10 minutes at 3,000 rotations per minute. After the plasma separation, red blood cells were washed four times with 3 mL of saline followed by stirring and centrifuging for 10 minutes at 3,000 rotations per minute. Washed red blood cells were supplemented with 2 mL of distilled water. The obtained hemolysate was divided into two samples for analysis of CAT and SOD in red blood cells. The analysis of CAT was carried out by monitoring of the fall in absorbance at 240 nm in a solution of hydrogen peroxide with phosphate buffer. The obtained values were expressed as U/mL. The minus sign ahead of the value was (can be) ignored. The analysis of SOD of red blood cells was performed by enzymatic kinetic method using commercial BIOREX kit. The obtained values were expressed as U/g of hemoglobin.

RESULTS

Statistical analysis of parameters and distribution superoxide dismutase, catalase and blood glucose

In Table 1, values are given (mean value and standard deviation) for parameters SOD, CAT, fasting and postprandial 2 h glucose (G0H and G2H) in study groups.

For the established normal distributions of SOD in the control group and in the group of obese individuals, t-test confirms a significant difference in mean values of this enzyme ($t = 3.53$, $p = 0.0014$). Uniform distribution in T2DM group refers to the systematic change of SOD after the treatment.

For the established normal distributions of CAT in the control group, the group of obese individuals and in the T2DM group before the treatment, using t-test founds no significant changes in the mean value of CAT.

Significant change in the structure of distribution of CAT in the T2DM group after the treatment also refers to the systematic change and to absolute differences in CAT with complementary groups. This difference is caused by treatment.

For the established normal distributions of G0H in the control group and in the group of and obese individuals, using t-test confirms a significant difference in mean values of G2H ($t = -3.09$, $p = 0.0044$).

The difference between mean values of G0H ($Z = 4.31$; $p = 0.000016 < 0.05$) and G2H ($Z = 3.31$; $p = 0.000856 < 0.05$) in the T2DM group before and after the treatment is significant (Signum test). Therapy had a significant effect.

Correlations of superoxide dismutase and catalase

In order to determine the nature of the oxidative stress, the starting point was the relationship between SOD as the independent variable, and CAT as the dependent variable. The following equations of linear regression and correlation coefficients were established and given in Figure 4.

It should be noted that analytical parameters of the regression line, i.e. free member, and the coefficient for the independent variable are very close (-160.6 for control group and -163.4 for obese individuals).

However, in the group of patients with newly diagnosed diabetes systematic change in the relation between SOD and CAT is occurring. The value of the free member (-90.07) changes drastically for about 44% (from initial ≈-160) while the coefficient for the independent variable changes the sign (-0.1782). Dependent systematic change

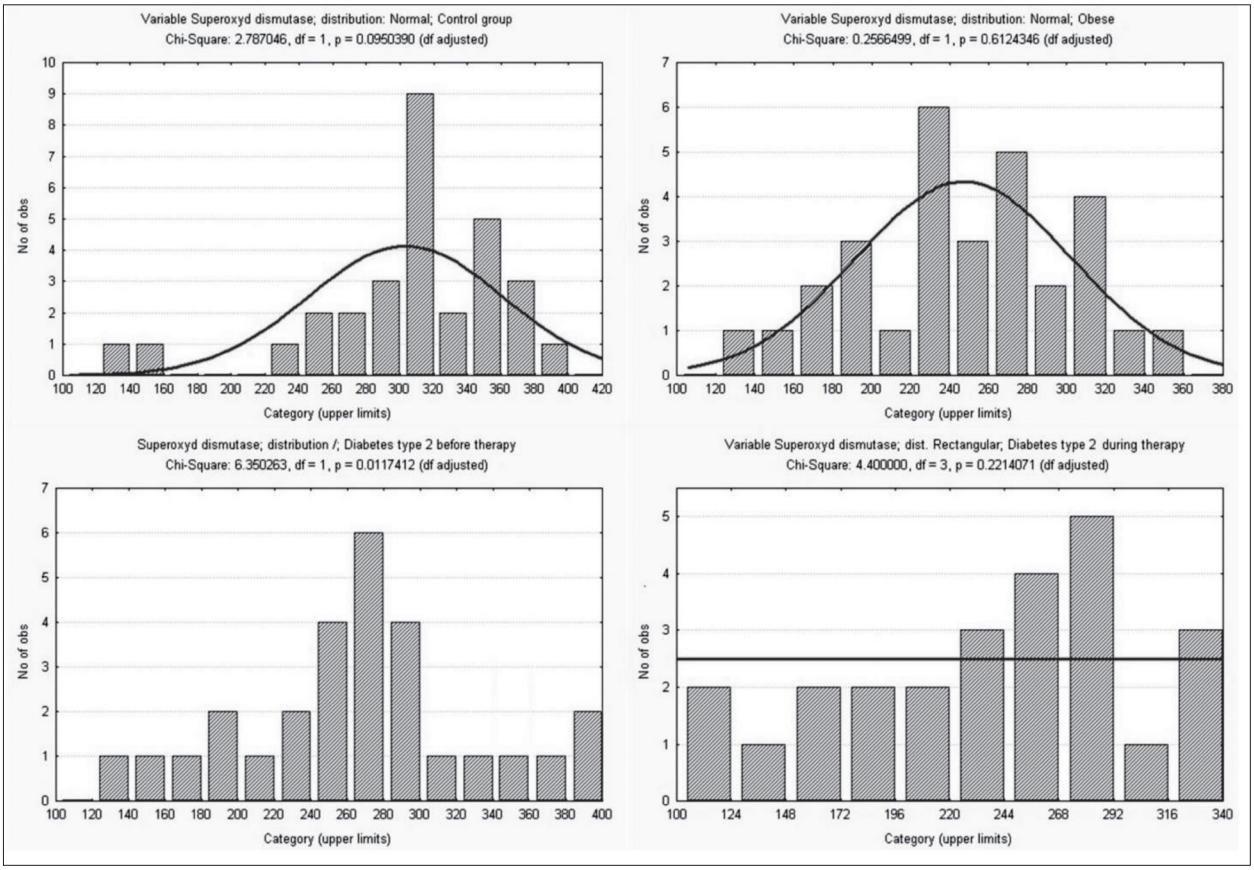

Figure 1. Distribution and verification of superoxide dismutase in control group, group of obese individuals without diabetes, type 2 diabetes patients at the baseline and after the treatment period

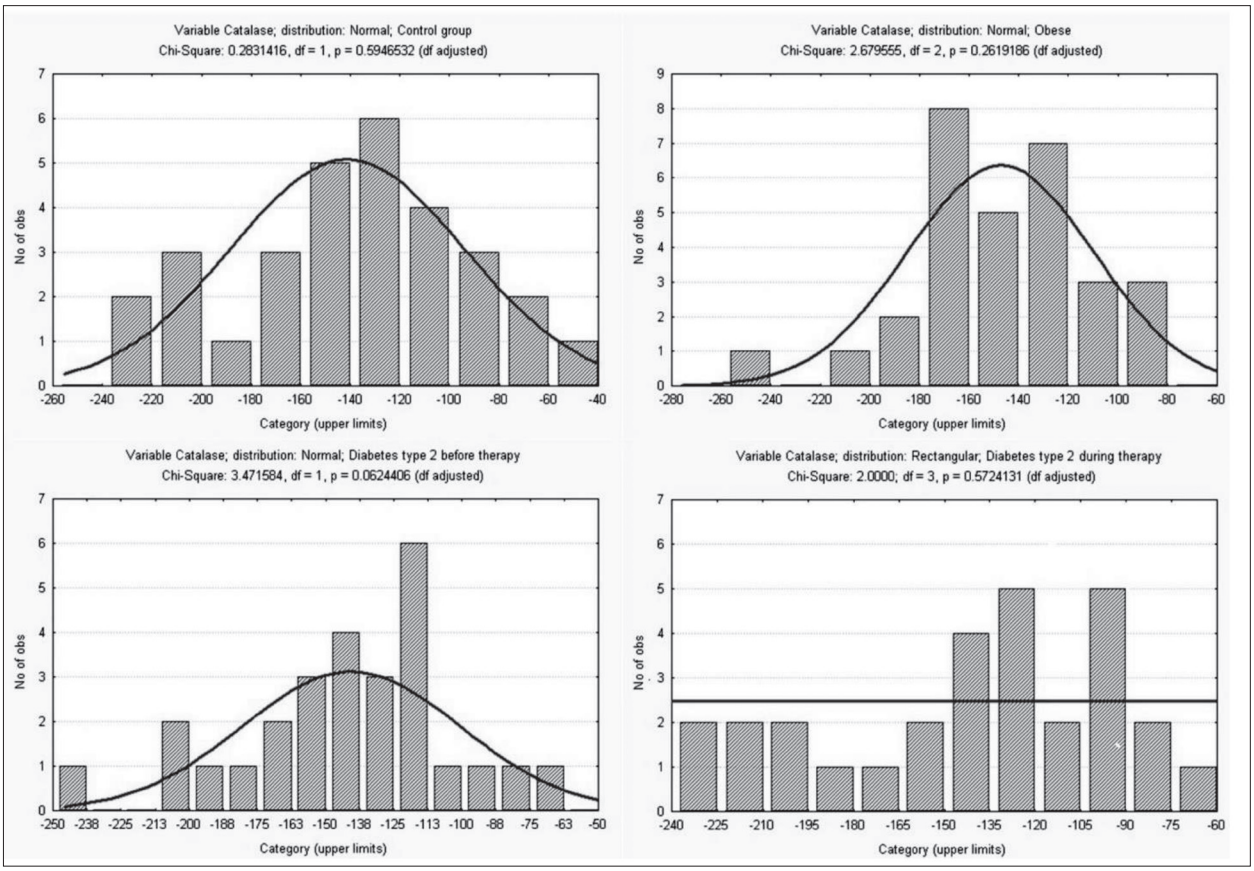

Figure 2. Distribution and verification of catalase in control group, group of obese individuals without diabetes, type 2 diabetes patients at the baseline and after the treatment period

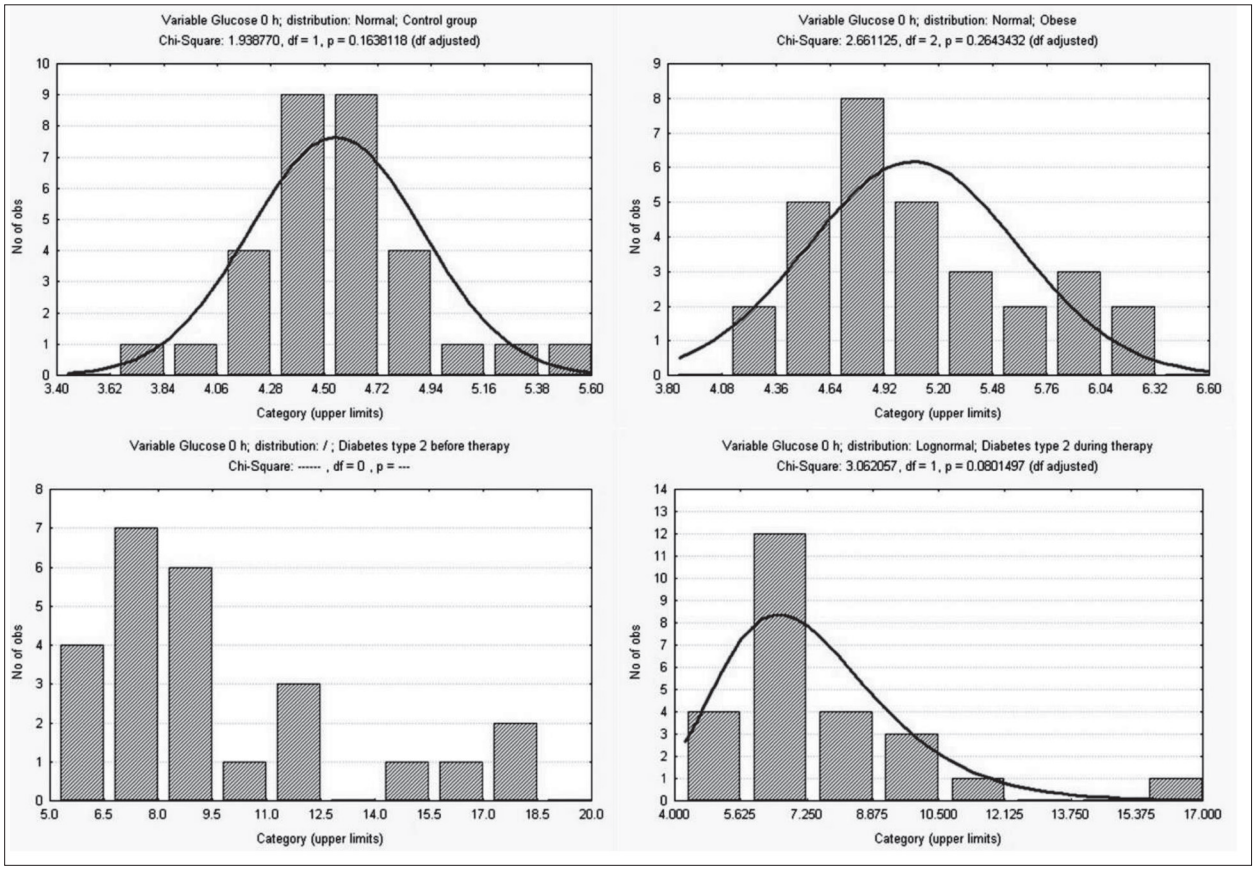

Figure 3. Distribution and verification of fasting glucose in control group, group of obese individuals without diabetes, type 2 diabetes patients at the baseline and after the treatment period

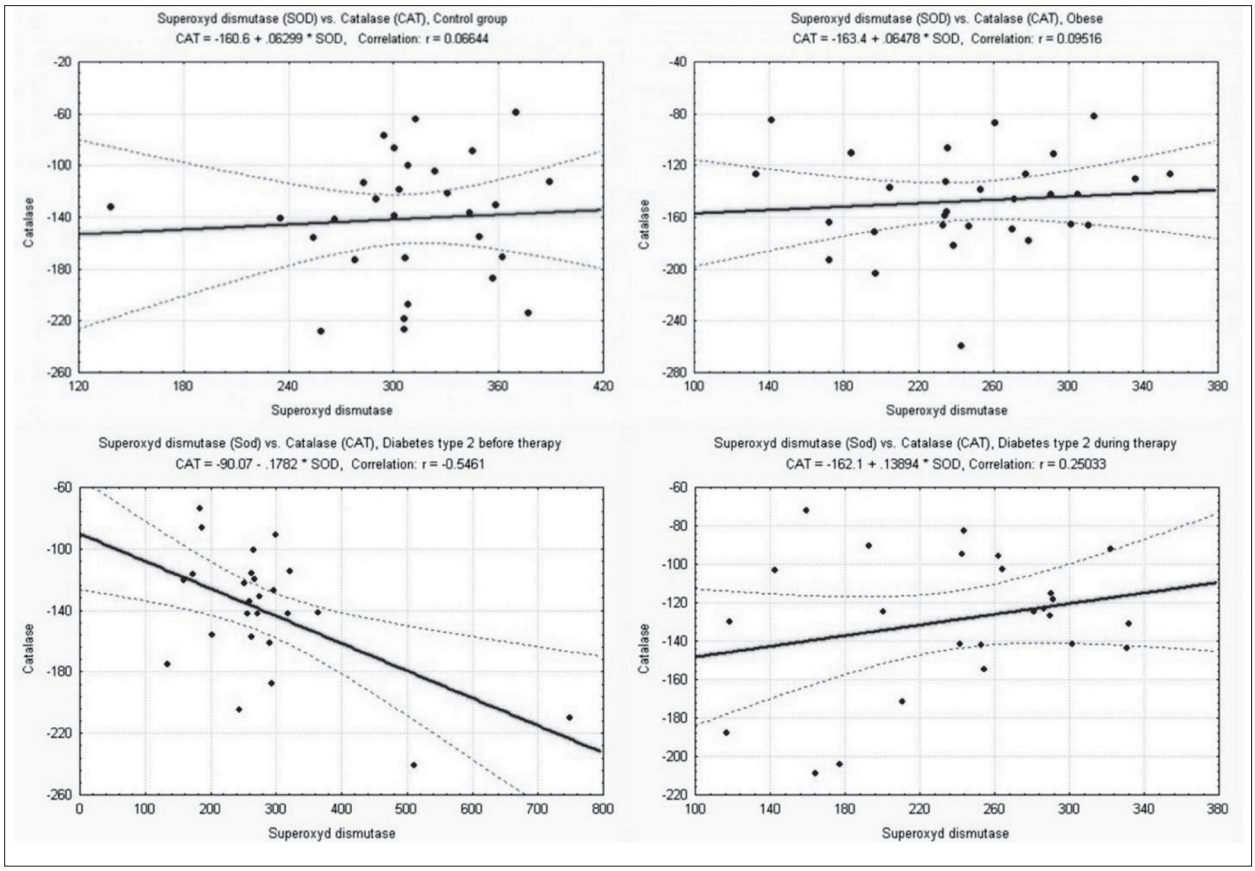

Figure 4. Equations, correlation coefficients, graphical display of lines of linear regressions of superoxide dismutase and catalase in control group, group of obese individuals without diabetes, type 2 diabetes patients at the baseline and after the treatment period

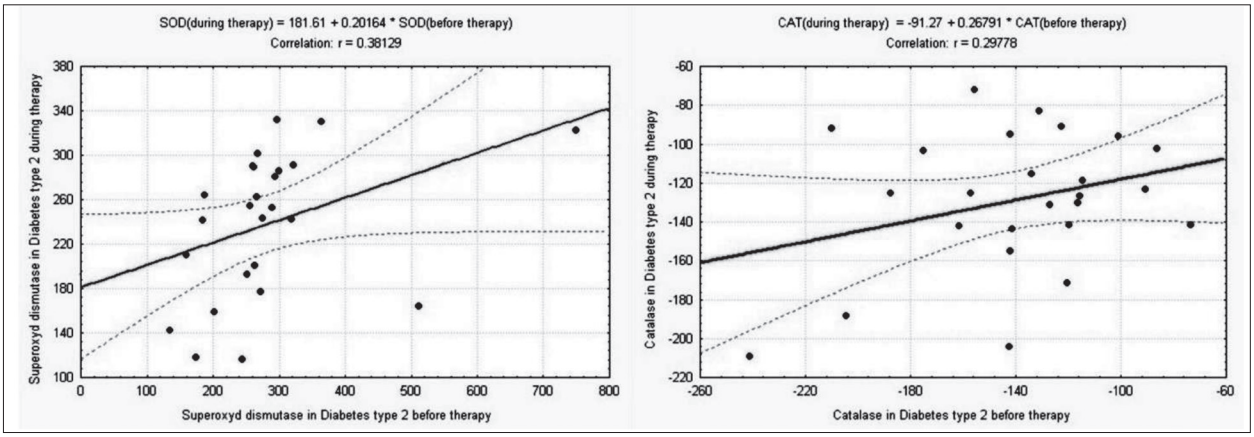

Figure 5. Effects of metformin therapy on mutual relationships between superoxide dismutase and catalase values

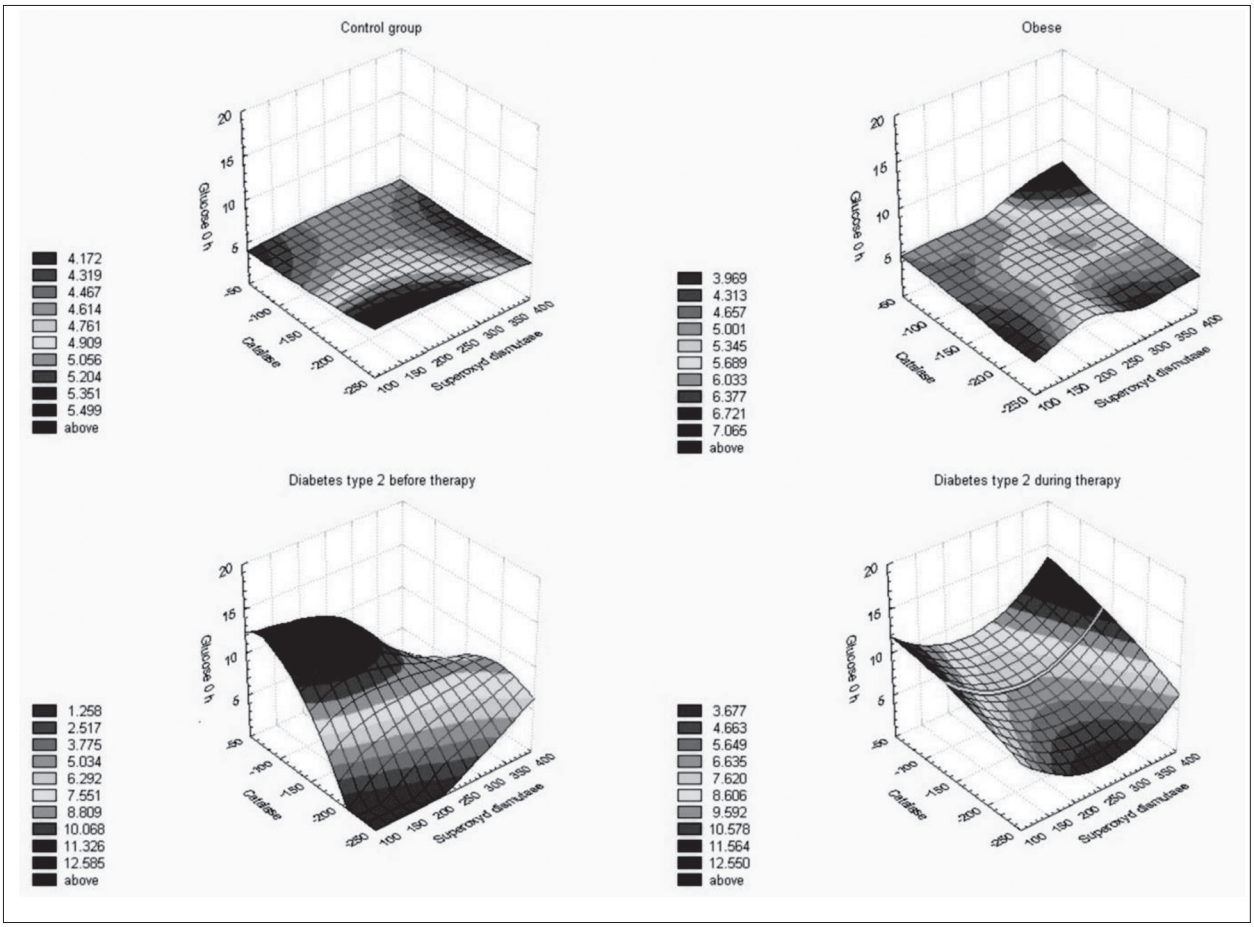

Figure 6. Two-dimensional G0H as dependent variable in the function of independent variables superoxide dismutase and catalase in control group, group of obese individuals without diabetes, type 2 diabetes patients at the baseline and after the treatment period

describes and validates the negative correlation coefficient (r = -0.5461).

The applied therapy in patients with diabetes returns the correlation parameters of SOD and CAT close to established among the control group and the group of obese individuals. The value of the free member of the regression line (-162.1) is close to the value in the control group and in the group of obese individuals (≈-160), so therapy eliminated difference of 44%, which was the result of the diabetes onset. The coefficient of the independent variable is positive again (+0.1389). The value of correlation coefficient of the enzyme after the treatment (r = +0.2503) points out that "moderate" systematic association in diabetes is reduced to "low" after the treatment.

The biggest difference in changes in values of correlation coefficients occurs in the group of T2DM patients before and after the treatment. Pearson's test declares this change as significant p = 0.0011, i.e. therapy significantly changes linear relationships of SOD and CAT. A more detailed insight into the effect of therapy linear regressions of SOD and CAT in T2DM group before and after the treatment is given in Figure 5. Directly proportional

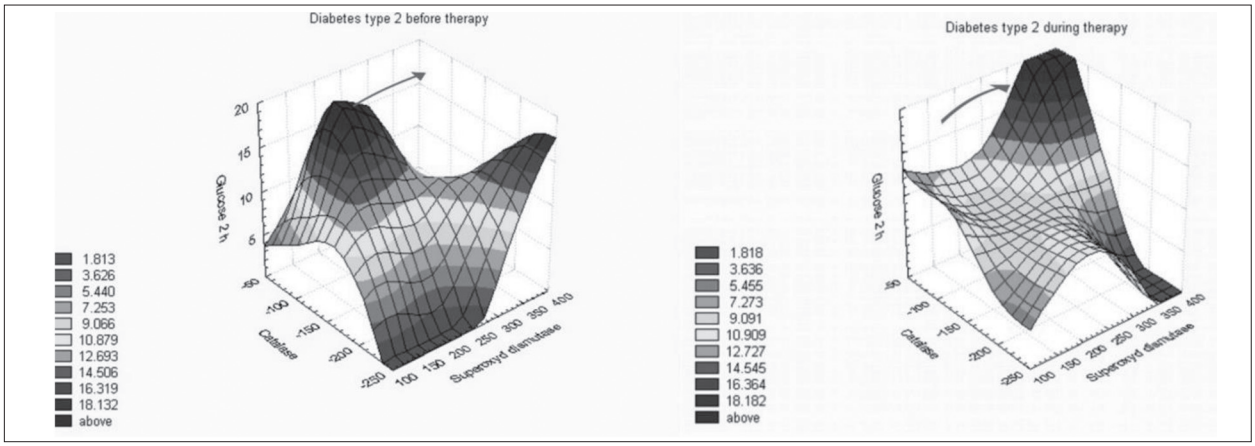

Figure 7. Two-dimensional postprandial 2-hour glucose as dependent variable in the function of independent variables superoxide dismutase and catalase in type 2 diabetes patients at the baseline and after the treatment period

relationships are more pronounced in SOD (r = +0.3812) than in CAT (r = +0.2977).

Low values of coefficients of the variable before the treatment (0.20164 for SOD and 0.26791 for CAT) (Figure 5) are excluding the possibility of the individual intense change in the value of enzymes caused by therapy.

Determining the interval of synergistic influence of superoxide dismutase and catalase on blood glucose levels

Specific and significant change in the correlations between SOD and CAT prior to the treatment, intrigues further analysis of paths through which enzymes influence the basic parameter of diabetes – blood glucose. The analysis continues with exploring of synergic influence of SOD and CAT primarily on the G0H. From statistical data the approximate two-dimensional function of independent variables (SOD and CAT) and dependent variable (G0H) is formed, which is shown in Figure 6. The onset of diabetes in obese individuals, in addition to drastic changes of coefficients in linear regression line equation of the relationship between SOD and CAT, the change of two-dimensional G0H function is expressed. The appearance of the glucose maximum on coordinates of independent variables SOD ≈ 150 and CAT ≈ -110 marked by values of dependent variable G0H > 10.

After treatment period, mild parabola determined by SOD with the minimum line at about SOD ≈ 250 which is in linear decrease in the function of CAT emerges. It is evident that the treatment is "calming" the maximum and that the inflection limit for CAT ≈ -135 (underlined in Figure 6).

When forming two complementary groups of T2DM patients before and after the treatment with regard to cut-off point of CAT ≈ -135, the group with 0 > CAT > -135 have mean G0H of 10.969, while the group with -135 > CAT >-∞ have mean G0H of 7.883. These values differ significantly based on analysis of variance (ANOVA) p = 0.03537 < 0.05 (df = 23, F = 4.9977).

The equation of the linear regression of SOD and CAT (CAT = -90.07 - 0.1782 × SOD) can be expressed in an inverse form in which CAT is independent variable and SOD is dependent variable. If we enter characteristic value of CAT = -135 in this linear regression equation, the assumed characteristic value SOD is obtained:

$$SOD = 48.33 - 1.674 \times CAT \rightarrow SOD = 48.33 - 1.674 \times -135 = 274.32 \approx 270$$

Adopted value SOD=270 is characteristic because it represents the approximate direction of propagation of minimum parabolic drawing, the axis of the two-dimensional function of G0H dependence in T2DM patients Diabetes type 2 (Figure 6, after the treatment).

However, although among low values SOD does not give a significant difference in G0H value by itself, a synergistic effect of factor (0 < SOD < 270) with factor (0 > CAT > -135) reveals a group of patients which has an exceptionally high G0H before the treatment with the mean value of G0H = 12.656 in contrast to the value of complementary group G0H = 7.7063. The analysis of variance for the declared intervals of value of the intervals of both enzymes (MANOVA test) points out the significant difference between groups with the significance threshold of p = 0.000424 < 0.01 < 0.05.

In search for a possible dynamic of influence of SOD and CAT factors on blood glucose levels, analysis of two-dimensional dependence of G2H in a function of independent variables (SOD and CAT) is continued. Graphics of approximate function for T2DM patients before and after the treatment are given in Figure 7. After the treatment, changes are occurring for a variable G2H - from the parabolic form (Figure 6) to the translation of maximum before the treatment into zones of high values of SOD, which are highlighted by arrows in Figure 7. However, these changes are not significant (MANOVA p = 0.0797 < 0.05!).

DISCUSSION

The activities of SOD, CAT and GPX constitute a first line antioxidant defense system, which plays a key role in the defense mechanisms in biological systems [12].

Controversial reports on changes in serum antioxidative enzyme activity of T2DM patients have been published [13]. Our study demonstrated very specific changes in value and distribution of CAT and SOD. CAT has proven to be the more stable enzyme, which did not change the systematic distribution with the onset of diabetes. The mean value of CAT after the onset of diabetes remained at the level similar to one in the control group and in the group of obese individuals without diabetes. Unlike CAT, SOD expressed the clear systematic change with the onset of diabetes. These changes in values of SOD most probably occur due to the faster and more unstable reaction of SOD molecules during the onset of diabetes.

In addition to biochemical changes in glucose levels, the incidence of diabetes caused disorder of dynamics of the relationship between SOD and CAT, through significant changes in their mutual correlations. The system of distribution of CAT and SOD is significantly changed after the metformin therapy. With the onset of diabetes CAT remained normally distributed but SOD lost the allocation system. It is assumed that the breakdown of SOD distribution system is regulatory, due to incomplete exhaustion of CAT and regulatory feedback mechanism of reduced production of hydrogen peroxide by SOD, in order to maintain the normal functioning of CAT. A study by Goth et al. [14], which found that individuals with CAT insufficiency have significantly lower levels of SOD, supports this assumption.

After the treatment period, distribution of both enzymes transforms into uniform. The equilibrium form of uniform distribution without expressed statistical module points out the role of treatment in supporting the balance of these enzymes. In doing so, it does not matter whether the values of enzymes are back to the control group level, or to the level of the group of obese individuals without diabetes, but it is important that the relationship of SOD and CAT returns to the relationship that existed before the onset of diabetes. Correlation of enzymes in the control group, in the group of obese individuals without diabetes, and in the group of T2DM patients after the treatment period was significantly same, and this correlation is significantly different in the group of T2DM patients at the baseline. According to the response to the metformin therapy, it appears that the functional relationship of enzymes is more important than their individual values. This indicates that exhaustion of SOD and CAT with mandatory disorder in their correlations can predict the development of T2DM. Heuristic search for values of critical exhaustion of the enzyme generated the value of CAT = -135 U/mL. T2DM patients at the baseline with CAT less negative than this cut-off point had significantly higher fasting plasma glucose than patients with CAT which was more negative than this cut-off value. Setting the cut-off value of SOD at 270 U/g of hemoglobin revealed that within the same group of patients, ones with lower levels of this enzyme do not have significantly higher values of fasting plasma glucose in comparison to patients with greater levels of SOD. This reflects the most stable level of antioxidant capacity and significance of CAT. However, synergistic effect of CAT and SOD is significant, so at the baseline, T2DM patients with lower level of SOD and lower activity of CAT have significantly higher levels of fasting plasma glucose in comparison with patients with higher SOD value and higher CAT activity. Observed synergistic relationship of enzymes is, at least to some extent, described by Lortz and Tiedge [15], who found that optimal protection of pancreatic β-cells from oxidative damage is provided by combined increased expression of both SOD and CAT. The exhaustion of compensatory mechanisms of CAT and/or its glycosylation in the group of patients with newly diagnosed T2DM likely leads to excessive accumulation of hydrogen peroxide and subsequent suppression of the SOD activity through the feedback mechanism. The suppression of SOD activity may lead to further damage of antioxidative enzymes by accumulated superoxide anions (insufficient elimination of these anions by SOD). However, established critical levels of enzymes and their synergy is losing significance after the additional oxidative load (postprandial state). Significantly superimposed levels of glucose are most probably caused by the insufficiency increase of antioxidant enzymes after a meal. In addition to the recovery correlation between enzymes and significant decrease in glucose levels, the metformin therapy has eliminated the existence of the critical interval of enzymes.

In our study, recovery of correlations between enzymes after the metformin treatment may be a result of short duration of diabetes, so this hypothesis should be tested in the larger population of T2DM patients and with a longer period of the follow-up.

Bakala et al. [16] were examining mitochondrial extracts from the liver of experimental animals and fond that CAT is the most vulnerable to glycosylation of all antioxidant enzymes. High levels of hydrogen peroxide are present in cells with sufficient quantity of GPX, but with reduced reserve of CAT [17]. These results are in accordance with ours, which report association between higher levels of glucose and lower levels of CAT activity, and even more pronounced association of higher glucose levels with combined presentation of lower levels of SOD and lower activity of CAT. Since CAT appears also as an anticancer target, the elucidation of mechanisms regulating its expression is an important issue [18].

Goth et al. [19] considered that elevated concentrations of hydrogen peroxide, due to reduced activity of CAT, can contribute to the oxidative damage of pancreatic β-cells, thus reducing the secretion of insulin and leading to the onset of diabetes and the increase in carotid intima media thickness [20]. New research by Goth et al. [21] reports low levels of CAT in T2DM patients but not in patients with type 1 diabetes. Mutual cooperation of antioxidative enzymes is also vital for normal functioning of the organism [22]. Alfa-lipoic acid (ALA) an essential co-enzyme for energy production in mitochondria, demonstrates substantial antioxidant properties and an effect on whole-body physiology like inhibition of glycation reactions and prevention of beta-cell destruction [23]. It has been used in several oxidative-stress models such as diabetes, ischemia-reperfusion injury, cataract, and neurodegenerative disorders.

The current findings suggest that α-lipoic acid is beneficial and thus should be considered for routine administration in patients with diabetes and peripheral neuropathy [24]. A study by Yang et al. [25] demonstrated that metformin might be effective for reducing blood glucose and promoting glucose uptake and utilization and that ALA might be effective for improving insulin sensitivity and activating insulin-signaling pathways. They demonstrated that ALA enhances hepatic insulin sensitivity and prevents the development of Non-alcoholic fatty liver disease, furthermore, ALA ameliorates glucose metabolism by modulating the insulin-signaling pathway.

CONCLUSION

Although significant differences in absolute values of CAT between groups were not observed, significant changes in linear correlation of CAT and SOD, in particular in the group of T2DM patients treated with the metformin therapy, are clearly visible. In cases of low CAT activity caused by physiological reserves exhaustion, followed by the hydrogen peroxide accumulation, which beside its toxic oxidative action, also inhibits the SOD activity through the feedback mechanism. Significantly higher fasting plasma glucose levels are found among group with low CAT activity and low SOD level comparing to the complementary group. Taking into account that CAT is the most vulnerable to glycosylation among all antioxidant enzymes, and that it has the highest metabolic turnover rate among all known enzymes due its physiological role in removing molecules of hydrogen peroxide (as the most stable oxidative compound), it seems that its activity is the key point of maintaining the oxidative-reduction balance in terms of glycemic control [26]. The question remains of the cause and the effect. If the disorder of enzymes relationship precedes diabetes development, then these conclusions could be used in the individual prevention of diabetes. However, in most cases we do not have the data on the diabetes onset and duration prior to its diagnosis. Longitudinal follow-up of values of these antioxidant enzymes in obese patients prone to diabetes could lead to an answer. Long-term monitoring of dynamics of these enzymes during diabetes duration may provide a more detailed insight into their function, which may not be identical as in the newly diagnosed T2DM patients. It remains an open question whether long-term treatment with metformin maintains correlation antioxidant enzymes relationships in the same way as is observed after the three-month treatment period. This may be an important issue because the primary therapeutic response is relationship synchronization between antioxidant enzymes, and not the impact on their individual absolute values, which are influenced by many other factors.

Conflict of interest: None declared.

REFERENCES

1. Fındıklı E, Camkurt M, İzci F, Karaaslan M, Fındıklı H, Sümer P, et al. Diagnostic value of malondialdehyde, superoxide dismutase and catalase activity in drug naïve, first episode, non-smoker generalized anxiety disorder patients. Clin Psychopharmacol Neurosci. 2018; 16(1):88–94.
2. Mohseni R, Arab Sadeghabadi Z, Goodarzi MT, Teimouri M, Nourbakhsh M, Razzaghy Azar M. Evaluation of Mn-superoxide dismutase and catalase gene expression in childhood obesity: its association with insulin resistance. J Pediatr Endocrinol Metab. 2018; 31(7):727–32.
3. Johansen JS, Harris AA, Rychly DJ, Ergul A. Oxidative stress and use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol. 2005; 4:5.
4. Kusano C, Ferrari B. Antioxidant defenses in diabetes mellitus: a clinical and molecular approach. Integr Obesity Diabetes. 2017; 3(5):1–7.
5. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharmaceutical Journal. 2016; 24(5):547–53.
6. Srivatsan R, Das S, Gadde R, Manoj Kumar K, Taduri S, Rao N, et al. Antioxidants and lipid peroxidation status in diabetic patients with and without complications. Arch Iran Med. 2009; 12(2):121–7.
7. Song F, Jia W, Yao Y, Hu Y, Lei L, Lin J, et al. Oxidative stress, antioxidant status, and DNA damage in patients with impaired glucose regulation and newly diagnosed type 2 diabetes. Clin Sci. 2007; 112(12):599–606.
8. Kajanachumpol S, Komindr S, MahaisiriyodomA. Plasma lipid-peroxide and antioxidant levels in diabetic patients. J Med Assoc Thai. 1997; 80(6):372–7.
9. Matkovics B, Kotorman M, Varga IS, Hai DQ, Salgo L, Novak Z. Pro-antioxidant and rheologic studies in the blood of type 2 diabetic patients. Acta Physiol Hung. 1997; 85(2):107–12.
10. Ngaski AA. Correlation of antioxidants enzymes activity with fasting blood glucose in diabetic patients in Sokoto, Nigeria. Journal of Advances in Medicine and Medical Research. 2018; 25(12):1–6.
11. Peuchant E, Delmas-Beauvieux MC, Couchouron A, Dubourg L, Thomas MJ, Perromat A, et al. Short term insulin therapy and normoglicaemia. Effects of erythrocyte lipid peroxidation in NIDDM patients. Diabetes Care. 1997; 20(2):202–7.
12. Ighodaro, OM, Akinloye OA. First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. Alexandria Journal of Medicine. 2018; 54(4):287–93.
13. Zhao JS, Jin HX, Gao JL, Pu C, Zhang P, Huang JJ, et al. Serum extracellular superoxide dismutase is associated with diabetic retinopathy stage in Chinese patients with type 2 diabetes mellitus. Dis Markers. 2018; 2018:8721379.
14. Goth L, Nagy T. Acatalasemia and diabetes mellitus. Arch Biochem Biophys. 2012; 525(2):195–200.
15. Lortz S, Tiedge M. Sequential inactivation of reactive oxigen species by combined overexpression of SOD isoforms and catalase in insulin production cells. Free Radic Biol Med. 2003; 34(6):683–8.
16. Bakala H, Hamelin M, Mary J, Borot-Laloi C, Friguet B. Catalase a target of glycation damage in rat liver mitochondria with aging. Biochim Biophys Acta. 2012; 1822(10):1527–34.
17. Lopez-Torres M, Perez-Campo R, Rojas C, Cadenas S, Barja G. Simultaneous induction of SOD, glutation reductase, GSH and ascorbate in liver and kidney correlates with survival during ageing. Free Radic Biol Med. 1993; 15(2):133–42.
18. Glorieux C, Sandoval JM, Dejeans N, Nonckreman S, Bahloula K, Poirel H, et al. Evaluation of potential mechanisms controlling the catalase expression in breast cancer cells. Oxid Med Cell Longev. 2018; 2018:5351967.
19. Goth L, Lenkey A, Bigler WN. Blood catalase deficiency and diabetes in Hungary. Diabetes Care. 2001; 24(10):1839–40.
20. Kaplar M, Nagy T, Góth L. Association of catalase gene rs769217 polymorphism with carotid intima-media thickness in diabetic patients. Int J Diabetes Metab Disord, 2018; 3(1):1–5.
21. Goth L, Nagy T, Paragh G, Kaplar M. Blood catalase activities, catalase gene polymorphisms and acatalasemia mutations in

Hungarian patients with diabetes mellitus. Glob J Obes Diabetes Metab Syndr. 2016; 3:1–5.
22. Gil D, Rodriguez J, Ward B, Vertegel A, Ivanov V, Reukov V. Antioxidant activity of SOD and catalase conjugated with nanocrystalline ceria. Bioengineering. 2017; 4(1).
23. Serhiyenko V, Serhiyenko L, Suslik G, Serhiyenko A. Alpha-lipoic acid: mechanisms of action and beneficial effects in the prevention and treatment of diabetic complications. MOJ Public Health. 2018; 7(4):174–8.
24. Agathos E, Tentolouris A, Eleftheriadou I, Katsaouni P, Nemtzas I, Petrou A, et al. Effect of α-lipoic acid on symptoms and quality of life in patients with painful diabetic neuropathy. J Int Med Res. 2018; 46(5):1779–90.
25. Yang Y, Li W, Liu Y, Li Y, Gao L, Zhao JJ. Alpha-lipoic acid attenuates insulin resistance and improves glucose metabolism in high fat diet-fed mice. Acta Pharmacol Sin. 2014; 35(10):1285–92.
26. Fournet M, Bonté F, Desmoulière A. Glycation damage: A possible hub for major pathophysiological disorders and aging. Aging Dis. 2018; 9(5):880–900.

Узајамно деловање антиоксидативних ензима – корелациони односи каталазе и супероксидне дисмутазе током развоја и лечења дијабетеса типа 2

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САЖЕТАК

Увод/Циљ Литературни преглед појединачних анализа вредности ензима каталазе или супероксидне дисмутазе код оболелих од дијабетеса типа 2 (ДТ2) нема изражену конзистентност. Уз уважавање резултата свих претходних студија, пошли смо од претпоставке да се при појави дијабетеса не мењају значајно појединачни квантитети наведених ензима, али да се значајно нарушава однос наведених ензима изражен кроз поремећај њихове динамичке равнотеже.

Методе Студија се састојала од четири групе (n = 30 за сваку групу): гојазне особе са поремећеним метаболизмом глукозе (субјекти са новодијагностикованим ДТ2) пре и у току метформинске терапије, гојазне особе са нормалном толеранцијом глукозе и контролне групе здравих нормалне телесне масе. Одговарајућа антропометријска мерења и лабораторијска испитивања биохемијских параметара и антиоксидативних ензима су спроведена код свих учесника.

Резултати Налаз наше студије доказује знатне промене вредности линеарних корелација ензима каталазе и супероксидне дисмутазе код болесника са новооткривеним ДТ2. Примењена терапија метформином враћа динамичку равнотежу ензима каталазе и супероксидне дисмутазе на ниво гојазних болесника пре појаве дијабетеса.

Закључак Примењена терапија метформином враћа динамички баланс ензима каталазе и супероксидне дисмутазе на ниво ензима гојазних болесника са нормалном гликорегулацијом, кроз реинтеграцију новог равнотежног система вредности ензима и расподела вредности ензима. Ови закључци важе само за почетне фазе лечења ДТ2.

Кључне речи: антиоксидативни ензими; гојазност; метаболизам глукозе; метформин

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prevalence and risk factors for Barrett's esophagus in patients with chronic gastroesophageal reflux disease

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SUMMARY

Introduction/Objective The most important complication of gastroesophageal reflux disease (GERD) is Barrett's esophagus (BE) and the development of esophageal adenocarcinoma. Prevalence of BE is 5–15% in patients with GERD symptoms.
The aim of the study was to investigate the prevalence and risk factors for BE in patients with chronic reflux symptoms. A prospective study was conducted in the Clinic of Gastroenterology, Niš Clinical Center.
Methods We included 676 patients with chronic reflux symptoms, who underwent esophagogastroduodenoscopy. The biopsy specimens were obtained in a four-quadrant fashion at intervals of 2 cm from the circumferential endoscopic Barrett's epithelium in the distal esophagus. BE was diagnosed by pathological examination.
Results Out of the total number patients with GERD, 92 were diagnosed with columnar-lined esophagus (CLE), the prevalence being 13.6%. Histological examination of biopsy from 92 patients with CLE revealed specialized intestinal metaplasia in 15 patients, with the prevalence of 2.22%. Compared to patients without BE, patients with BE were older and more commonly male. Univariable analyses showed that hiatal hernia and *Helicobacter pylori* infection were two significant risk factors for the onset of esophagitis. The age and the presence of reflux symptoms were associated with the presence of BE. Older age could be considered a significant risk factor for the development of BE and GERD.
Conclusion Prevalence of biopsy-proven BE and CLE in Serbia was 2.22% and 13.6%, respectively, in patients with GERD symptoms.
Keywords: Barrett's esophagus; gastroesophageal reflux disease; chronic reflux symptoms

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a long-term condition where stomach contents come back up into the esophagus resulting in either symptoms or complications. GERD is mild acid reflux that occurs at least twice a month, or moderate to severe acid reflux that occurs at least once a week. In 20% of the population, symptoms last longer than one week. The prevalence of GERD significantly varies among different populations. The prevalence of all forms of GERD is 40%, the weekly symptoms have 14% of the population, and the daily symptoms range 4–7% [1]. Peptic esophagitis, reflux esophagitis and erosive esophagitis, erosive reflux disease (ERD) are synonyms for the subgroup of GERD patients with histopathological changes of esophageal mucosa that usually correlate with the symptoms of acid reflux content. Non-erosive reflux disease (NERD) includes the group of patients with symptomatic GERD who have no macroscopic mucosal changes noticed on the esophagogastroduodenoscopy. It is estimated that 50–70% of patients with GERD have NERD. Symptoms and signs of esophageal reflux disease can be varying intensity and are not always in correlation with the severity of esophageal damage [2].

Barrett's esophagus (BE) is a consequence of chronic GERD, that predisposes the development of esophageal adenocarcinoma (EAC) [3]. Endoscopically, the prevalence of BE has been estimated at 1–2% in all patients who underwent upper endoscopy for any indication, and anywhere from 5% to 15% in patients with symptoms of GERD. Among the malignant tumors of the esophagus, the incidence of Barrett's adenocarcinoma is increasing. The incidence of EAC has been three to four times higher in the last two decades. It is believed that the main reason for this high percentage of Barrett's adenocarcinoma is related to an increased incidence of BE, which shows a close causal relationship with GERD [4]. However, not all patients with gastroesophageal reflux and erosive esophagitis will develop BE, and all patients with BE do not have a history of gastroesophageal reflux. At least 25% of patients with BE do not have a history of GERD. In many patients with reflux esophagitis, treatment leads to the regeneration of the mucosa. Some patients will develop BE with an increased risk of developing EAC. There are many risk factors that can

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contribute to the development of BE, which is the subject of many studies in the world [5, 6].

The esophagus lined with columnar epithelium (CLE) and BE are the conditions in which stratified squamous epithelium is continuously replaced by a cylindrical epithelium from an esophageal-gastric junction. BE is characterized by the presence of specialized intestinal metaplasia (SIM). As SIM is part of the definition and is the epithelial type associated with cancer, obtaining biopsies from the columnar lined distal esophagus is mandatory. The sensitivity and positive predictive values of standard upper endoscopy for diagnosing BE have been reported as 82% and 34%, respectively [7]. Guidelines of the American College of Gastroenterology state that every patient with gastroesophageal reflux symptoms should at least once in his/her lifetime be referred for BE screening endoscopy. Patients with SIM in CLE are currently advised to undergo a periodic endoscopic surveillance to detect progression to dysplasia at an early, potentially curable stage. New techniques such as chromoendoscopy and magnification endoscopy have been tried to improve recognition of SIM [4].

The aim of this study was to determine the prevalence and possible risk factors of BE in patients with chronic reflux symptoms.

METHODS

A prospective study conducted at the Clinic of Gastroenterology, Niš Clinical Center, included 676 patients with chronic reflux symptoms and all underwent esophagogastroduodenoscopy. The symptoms are defined as the presence of heartburn and regurgitation at least three times a week for one year. The questionnaire was completed by every patient; the questionnaire included information on age, sex, occupation, as well as the following criteria: primary referral symptoms, frequency of GERD symptoms, acid test, extraesophageal symptoms. Patients with a history of documented peptic disease, gastric or esophageal surgery, and those with motor disorders such as achalasia, diffuse esophageal spasm, or scleroderma, were excluded. Gastroesophageal junction (GEJ) is defined as the beginning of the proximal limit of gastric mucosal folds (Figure 1). CLE was identified as a columnar epithelium over 1 cm from the GEJ which had a reddish color and a velvety texture that could be easily distinguished from the normal pale and glossy esophageal squamous epithelium. The length of the CLE was estimated by subtracting the distance from the incisors to the squamocolumnar junction (Z-line) from the distance between the incisors and the GEJ (Figure 2). The patients were classified as short-segment BE (SSBE) if the length of the columnar appearing mucosa was less than 3 cm above the GEJ, and long-segment BE (LSBE) if the length of the columnar mucosa was equal to or greater than 3 cm. The diagnosis of BE is based on the presence of endoscopic findings compatible with columnar epithelium in the distal esophagus and confirmed by the presence of SIM on biopsies (Figure 3).

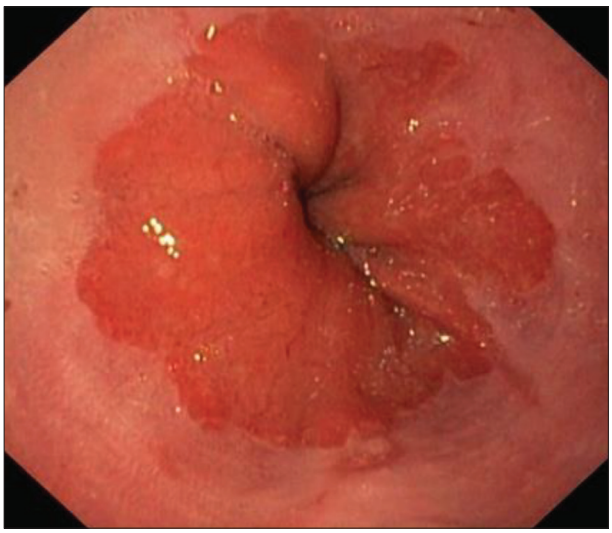

Figure 1. Endoscopic appearance of normal gastroesophagel junction; note that the squamocolumnar line corresponds with proximal extent of the gastric folds

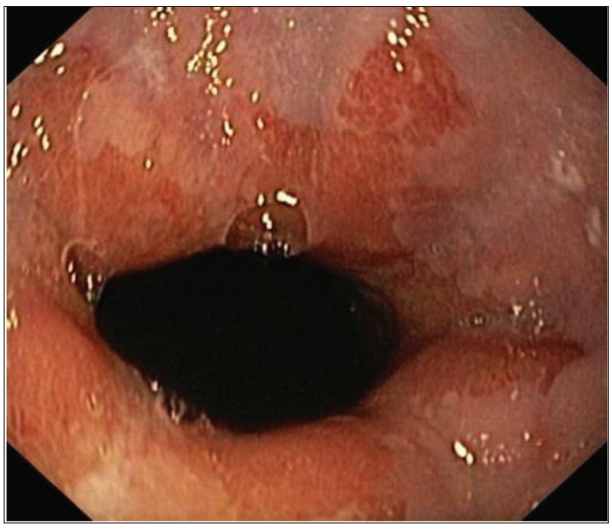

Figure 2. Salmon-colored mucosa is seen extending proximal to the gastroesophagel junction consistent with Barrett's esophagus

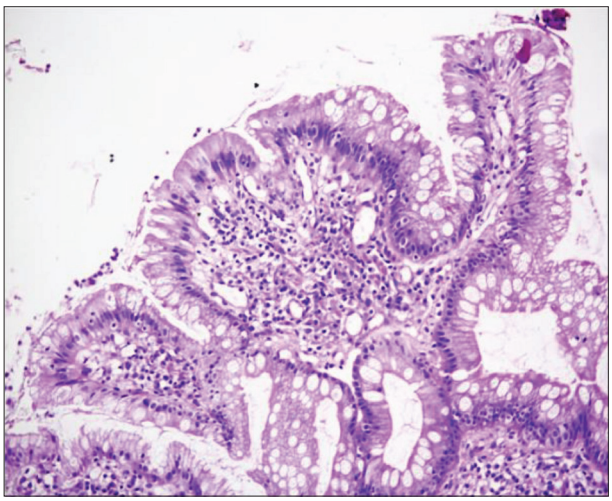

Figure 3. Histological appearance of Barrett's epithelium; intestinalized mucosa with branching pits and goblet cells (H&E, ×20)

The study protocol was approved by the local ethics committee and all patients gave their informed consent to be included. All the patients were fully informed of the study protocol and agreed to undergo upper gastrointestinal endoscopy.

All upper endoscopies were performed using a GIF100 or GIF130 video endoscope (Olympus, Lake Success, NY, USA). Macroscopic mucosal changes of the distal esophagus were measured on the basis of the distance from the Z line, and mucosal damage was classified according to the Los Angeles classification of reflux esophagitis [8].

The presence of a hiatal hernia and its size was determined in all the patients during the withdrawal of the endoscope and was measured in centimeters. We investigated the presence of *Helicobacter pylori* infection in all the patients by using pathology and rapid urease test.

The biopsy specimens were obtained in a four-quadrant fashion at intervals of 2 cm from the circumferential endoscopic Barrett's epithelium in the distal esophagus. In patients with small islands or irregular tongues of columnar appearing mucosa, at least two specimens were obtained within the abnormal-appearing mucosa at intervals of 1cm from the GEJ to the proximal extent of the abnormality. All biopsy specimens were stained with hematoxylin and eosin and with Alcian blue (pH 2.5) stain.

Statistical analysis

The processing of the obtained data was made using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). The data was processed using standard descriptive statistical methods (mean value, standard deviation, and percentage representation). The results were analyzed using the appropriate tests depending on the size of the group, type of mark, and type of distribution. We used the Student's t-test for continuous variables and χ^2 test for categorical variables, in comparative analyses. A univariate analysis was performed to determine the variables independently associated with the risk of BE. A p-value < 0.05 was considered statistically significant.

RESULTS

Patient with gastroesophageal reflux disease

The average age of subjects with the symptoms of reflux disease was 50 ± 13 years. There were 381 men (56.36%) and 295 women (43.64%). Based on endoscopic findings, patients were divided into two groups: the NERD group included 403 (59.61%) patients, and the ERD group included 273 patients (40.39%). Esophagitis A grade was found in 64.44%, B grade in 26.66%, and C grade in 8.88% of the ERD group patients. Esophagitis D grade was not found. The mean age of patients in both groups did not differ significantly (p = 0.07). The percentage of respondents by sex was approximately the same. Of the clinical manifestations of reflux disease, the heartburn symptom significantly correlates with ERD (p = 0.013). Heartburn was equally represented in the groups compared to daytime. In both groups of patients, heartburn was more frequent during the day (ERD, p = 0.00001; NERD, p = 0.00001), while fewer patients in both groups had heartburn at night. The symptom of regurgitation was more frequent in the NERD group in 222 (55.08%) patients, but without statistical significance. Hiatal hernia was more frequent in the ERD group, with a statistically significant (p = 0.001). *H. pylori* infection was significantly higher in NERD patients, 24.81% (n = 100). There was no correlation between the presence of *H. pylori* infection and the existence of reflux symptoms (Table 1).

Table 1. Background characteristics of the study groups

Characteristics	NERD (n = 403)	ERD (n = 273)	p-value
Age	49 ± 15	52 ± 17	0.07
Sex Male Female	 220 (54.59%) 183 (45.41%)	 161 (58.97%) 112 (41.03%)	0.30
Hiatal hernia Yes No	 91 (22.58%) 312 (77.42%)	 160 (58.61%) 113 (41.39%)	0.001
RUT Yes No	 100 (24.81%) 303 (75.19%)	 86 (31.5%) 187 (68.5%)	0.05
Heartburn	239 (59.3%)	190 (69.58%)	0.013
Regurgitation	222 (55.09%)	158 (57.87%)	0.54

NERD – non-erosive reflux disease; ERD – erosive reflux disease; RUT – rapid urease test

Prevalence of columnar-lined esophagus

Of all the patients with GERD, 92 had CLE, with the prevalence of 13.6%. Sixty-five patients were found to have normal endoscopy results, and 27 had erosive esophagitis (χ^2 = 27.39; p = 0.001). On endoscopic examination of all 92 patients, 35% had circumferential CLE, 34% had tongue-like extensions, and 31% isolated islands. A short CLE segment was found in 56% of the patients, and a long CLE segment was found in 13% of the patients.

Prevalence of Barrett's esophagus

Histological examination of biopsy from 92 patients with CLE revealed SIM in 15 patients, with the prevalence of 2.22% in our study. Of the 15 patients with BE, nine patients were found to have a long BE segment and six had a short BE segment. The average age of patients with BE was 59 ± 15 years, and 12 of them (80%) were male. The percentage of patients with CLE who had SIM was 16.3%; this was more frequent with a long CLE segment. The largest number of patients did not have erosive changes in the esophagus during endoscopy (87%), and the hiatal hernia was noticed in 80% of patients with BE (Table2).

Prevalence of Barrett's esophagus in gastroesophageal reflux disease

Compared to patients without BE, patients with BE were older and more commonly men, with statistical significance

Table 2. Predictors of specialized intestinal metaplasia or Barrett's esophagus

Characteristics	No metaplasia (n = 77)	Metaplasia (n = 15)	p-value
Age	49 ± 12	59 ± 15	0.001
Male Female	59 (76.62%) 18 (23.38%)	12 (80%) 3 (20%)	0.61 0.58
Heartburn	53 (68.83%)	2 (13.33%)	0.004
Regurgitation	19 (24.68%)	10 (66.67%)	0.12
NERD	52 (67.53%)	13 (86.67%)	0.34
ERD	25 (32.47%)	2 (13.34%)	0.25
Hiatal hernia	40 (51.95%)	12 (80%)	0.17
CLE Short segment Long segment	 47 (61.04%) 3 (3.89%)	 6 (33.34%) 9 (53.34%)	 0.29 0.005

NERD – non erosive reflux disease; ERD – erosive reflux disease; CLE – the esophagus lined with columnar epithelium

Table 3. Background characteristics of the study groups

Characteristics	BE (n = 15)	Without BE (n = 661)	p-value
Age	59 ± 15	49 ± 15	0.001
Male Female	12 (80%) 3 (20%)	372 (56.28%) 289 (43.72%)	0.06
Heartburn	2 (13.33%)	414 (62.63%)	0.04
Hiatal hernia Yes No	 12 (80%) 3 (20%)	 244 (36.91%) 417 (63.09%)	 < 0.05
RUT Yes No	 4 (26.66%) 11 (73.34%)	 182 (27.53%) 479 (73.47%)	 0.43

BE – Barrett's esophagus; RUT – rapid urease test

(p = 0.001). The symptom of heartburn was the dominant symptom, statistically occurring more frequently in patients with BE (p = 0.04). The univariate analyses showed that hiatal hernia and *H. pylori* infection were the most significant risk factors for the onset of esophagitis. The age and the presence of reflux symptoms are associated with the presence of BE (Table3).

DISCUSSION

In previous decades, the lower part of the esophagus and cardia have been in the focus of extensive research. The reason for this is a dramatic increase in the incidence of adenocarcinoma of the esophagogastric junction. In comparison, the incidence of GERD and BE as one of its complications was also noticed. Some data indicate a 10-fold increase in the incidence of Barrett's esophagus in Western European countries over the last few decades. Barrett's metaplasia is considered an intermediary event in the development of EAC [9].

In our study, the average age of subjects with symptoms of reflux disease was 50 ± 13. Almost 60% of patients with GERD did not have endoscopic signs of esophagitis, which is similar to those of Western countries, which shows that 60–70% of patients with typical reflux symptoms do not have damage of esophageal mucosa during endoscopy. In both groups, there were more male than female patients, though without statistical significance. Male sex has been reported to be an independent risk factor for esophagitis. Different parietal cell mass, lower esophageal function or body mass index between sexes have been proposed as possible causes to explain the sex effect [10]. Kumar et al. [11] show the prevalence of the male sex in patients with GERD.

Of the clinical manifestations of GERD, the heartburn symptom was statistically more frequent in the ERD group compared to the NERD group (p = 0.013), but there was no statistically significant association of heartburn symptoms with the degree of esophagitis. GERD symptoms have been inconsistently correlated with endoscopic findings of eosinophilic esophagitis in different studies, some of which favor such correlation, though not with all reflux symptoms, and some argue against it [12].

Hiatal hernia is present in 37.13% of patients with GERD. In the ERD group, the hiatal hernia is present in 58.61% of the patients. We found that the presence of hiatal hernia is a strong risk factor for esophagitis (p = 0,001) [13].

The relationship between *H. pylori* and GERD infection is relatively unclear. H. pylori gastritis can lead to acid hyposecretion and loss of symptoms of burning sensation [14]. In our study, *H. pylori* infection was statistically more common in the NERD than in the ERD group (p = 0.04). We did not find a statistically significant relationship between the presence of *H. pylori* infection and the presence of typical reflux symptoms.

Of all patients with GERD, suspected CLE was found in 92% of patients, representing prevalence of 13.6% of patients with GERD. Sixty-five patients were in the NERD group, and 27 in the ERD group. (χ^2 = 27.39; p = 0.001). Of the 92 patients with suspected CLE revealed, SIM was present in 15 patients, with the prevalence of 2.22%. The prevalence of BE worldwide is different; it is assumed to be higher in the western than in the eastern countries of the world. Westhoff et al. [15] showed a prevalence of 13.2%. Ronkainen et al. [16] showed a prevalence of 2.3% in Sweden, while Kim et al. [17] show a prevalence of less than 1% in Korea. In our study, BE was more common in men (80%) than in patients without BE (56.02%). BE prevalence was statistically more common in men than in women (p < 0.05). Lin et al. [18] in their study showed that 14% of women had BE, compared to 23% of men with BE (p < 0.05). Male sex has been reported to be a risk factor for BE. Age has been also considered a risk factor for it. Edelstein et al. [19] noted that risk of BE increased with increased age. In our study, patients with BE were significantly older than those without BE (p = 0.001). In clinical manifestation, we found a significant difference for heartburn between patients with BE and those without BE, which was more evident in patients with BE. The symptoms of reflux in our study was a good predictor of the risk for BE (p = 0.04), which is in a line with another study. Hak et al. [20] in their study showed that the duration of reflux symptoms is longer in patients with BE than in those without it. In our study, we noticed a significant difference in the existence of hiatal hernia between the

groups – hiatus hernia was more common in patients with BE. Herrera et al. [21] in their study showed that hiatus hernia is independently associated with the presence of BE.

In our study, we did not find that eosinophilic esophagitis is a predictor for the appearance of BE. Different morphological types of BE are not a risk factor for BE. The CLE length is a risk factor for BE. The CLE length was 3 cm in patients with BE, compared to 1.8 cm in patients without BE ($p = 0.001$). Okita et al. [22], as well as others, also proved that the long segment of BE is a predictor of SIM in the histological examination [23, 24, 25]. In our study, we did not show the presence of dysplasia in any of the patients with BE.

In conclusion, the prevalence of endoscopically suspected CLE in GERD patients is 13.6%. The prevalence of histologically proven BE was 2.22% in patients with GERD in our area. The presence of hiatal hernia, reflux symptoms, and long segment of CLE are independently associated with the presence of BE. Older age could be considered a significant risk factor for the development of BE and GERD.

CONCLUSION

A large number of studies have noted that most patients who have endoscopically suspected BE did not have SIM on histological samples. Multicenter studies are required for determining the epidemiology of BE more precisely, after which a cost-effective strategy for BE screening and surveillance can be developed. Studies should be carried out to determine endoscopic predictors, which can be used as surrogate markers for the histological BE, so that only patients with this predecessor are subjected to biopsy.

REFERENCES

1. Dent J, El-Serag HB, Wallande MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2005; 54(5):710–17.
2. Armstrong D. Systematic review: persistence and severity in gastroesophageal reflux disease. Aliment Pharmacol Ther. 2008; 28(7):841–53.
3. Labenz J, Koop H, Tannapfel A, Kiesslich R, Hölscher AH. The epidemiology, diagnosis and treatment of Barrett's carcinoma. Dtsch Arztebl Int. 2015; 112(13):224–34.
4. Shaheen NJ, Falk GW, Iyer PG, Gerson LB. ACG clinical guideline: Diagnosis and management of Barrett's esophagus. Am J Gastroenterol. 2016; 111(1):30–51.
5. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. Prevalence of Barrett's esophagus in the general population: An endoscopic study. Gastroenterology. 2005; 129(6):1825–31.
6. Fan X, Snyder N. Prevalence of Barrett's esophagus in patients with or without GERD symptoms: role of race, age, and gender. Dig Dis Sci. 2009; 54(3):572–7.
7. Spechler SJ, Souza RF. Barrett's esophagus. N Engl J Med. 2014; 371(9):836–45.
8. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease (GERD) – a global evidence-based consensus. Am J Gastroenterol. 2006; 101(8):1900–20.
9. Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut. 2014; 63(1):7–42.
10. Rubenstein JH, Mattek N, Eisen G. Age and sex specific yield of Barrett's esophagus by endoscopy indication. Gastrointest Endosc. 2010; 71(1):21–7.
11. Kumar S, Sharma S, Norboo T, Dolma D, Norboo A, Stobdan T, et al. Population based study to assess prevalence and risk factors of gastroesophageal reflux disease in a high altitude area. Indian J Gastroenterol. 2011; 30(3):135–43.
12. Koek GH, Sifrim D, Lerut T, Janssens J, Tack J. Multivariate analysis of the association of acid and duodeno-gastro-oesophageal reflux exposure with the presence of oesophagitis, the severity of oesophagitis and Barrett's oesophagus. Gut. 2008; 57(8):1056–64.
13. Jones MP, Sloan SS, Rabine JC, Ebert CC, Huang CF, Kahrilas PJ. Hiatal hernia size is the dominant determinant oesophagitis presence and severity in gastroesophageal reflux disease. Am J Gastroenterol. 2001; 96(6):1711–7.
14. Rubenstein JH, Inadomi JM, Scheiman J, Schoenfeld P, Appelman H, Zhang M, et al. Association between helicobacter pylori and Barrett's oesophagus, erosive esophagitis and gastroesophageal reflux symptoms. Clin Gastroenterol Hepatol. 2014; 12(2):239–45.
15. Westhoff B, Brotze S, Weston A, McElhinney C, Cherian R, Mayo MS, et al. The frequency of Barrett's esophagus in high-risk patients with chronic GERD. Gastrointest Endosc. 2005; 61(2):226–31.
16. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology. 2005; 129(6):1825–31.
17. Kim JH, Rhee PL, Lee JH, Lee H, Choi YS, Son HJ, et al. Prevalence and risk factors of Barrett's esophagus in Korea. J Gastroenterol Hepatol. 2007; 22(6):908–12.
18. Lin M, Gerson LB, Lascar R, Davila M, Triadafilopoulos G. Features of gastroesophageal reflux disease in women. Am J Gastroenterol. 2004; 99(8):1442–7.
19. Edelstein ZR, Bronner MP, Rosen SN, Vaughan TL. Risk factors for Barrett's esophagus among patients with gastroesophageal reflux disease: a community clinic-based case-control study. Am J Gastroenterol. 2009; 104(4):834–42.
20. Hak NG, Mostafa M, Salah T, El-Hemaly M, Haleem M, Abd El-Raouf A, et al. Acid and bile reflux in erosive reflux disease, non-erosive reflux disease and Barrett's esophagus. Hepatogastroenterology. 2008; 55(82-83):442–7.
21. Herrera Elizondo JL, Monreal Robles R, García Compean D, González Moreno EI, Borjas Almaguer OD, Maldonado Garza HJ, et al. Prevalence of Barrett's esophagus: An observational study from a gastroenterology clinic. Rev Gastroenterol Mex. 2017; 82(4):296–300.
22. Okita K, Amano Y, Takahashi Y, Mishima Y, Moriyama N, Ishimura N, et al. Barrett's esophagus in Japanese patients: its prevalence, form, and elongation. J Gastroenterol. 2008; 43(12):928–34.
23. Spechler SJ. Barrett esophagus and risk of esophageal cancer: a clinical review. JAMA. 2013; 310(6):627–36.
24. Bhat SK, McManus DT, Coleman HG, Johnston BT, Cardwell CR, McMenamin U, et al. Oesophageal adenocarcinoma and prior diagnosis of Barrett's oesophagus: A population-based study. Gut. 2015; 64(1):20–5.
25. Pohl H, Sirovich B, Welch HG. Esophageal adenocarcinoma incidence: are we reaching the peak? Cancer Epidemiol Biomarkers Prev. 2010;19(6):1468–70.

Преваленца и фактори ризика за настанак Баретовог једњака код болесника са хроничном гастроезофагеалном рефлуксном болешћу

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САЖЕТАК

Увод/Циљ Најважнија компликација гастроезофагеалне рефлуксне болести (ГЕРБ) јесте појава Баретовог једњака (БЈ) и настанак аденокарцинома. Преваленца БЈ је од 5 до 15% код болесника са симптомима ГЕРБ-а. Циљ ове студије био је испитивање преваленце и ризичних фактора за настанак БЈ код болесника са хроничним симптомима рефлукса. Истраживање је спроведено у Клиници за гастроентерологију Клиничког центра у Нишу.

Методе Укључено је 676 болесника са хроничним рефлуксним симптомима, којима је урађена езофагогастродуоденоскопија. Биопсије су узимане из четири квадранта у дисталном делу једњака, на удаљености од 2 *cm* од ендоскопски суспектног БЈ. БЈ је дијагностикован патолошким прегледом.

Резултати Од укупног броја болесника са ГЕРБ-ом, суспектан БЈ је нађен код 92 болесника, што чини преваленцу од 13,60% у нашој студији. Након хистолошког испитивања биопсије суспектног БЈ нађена је специјализована интестинална метаплазија код 15 болесника, са преваленцом од 2,22%. У поређењу са болесницима без БЈ, болесници са БЈ су старији, чешће мушкарци, у оба параметра са статистичком значајношћу. Хијатална хернија и инфекција бактеријом *Helicobacter pylori* су два значајна фактора ризика за настанак езофагитиса. Старост и присуство симптома рефлукса су повезани са присуством БЈ. Старији узраст може представљати значајан фактор ризика за развој БЈ и ГЕРБ-а.

Закључак Преваленца хистолошки доказаног БЈ и суспектног БЈ у Србији је била 2,22%, а 13,60% код болесника са симптомима ГЕРБ-а.

Кључне речи: Баретов једњак; гастроезофагеална рефлуксна болест; хронични рефлуксни симптоми

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Assessment of quality of life and physical and mental health in children and adolescents with coeliac disease compared to their healthy peers

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SUMMARY

Introduction/Objective Strict gluten-free diet for life is the only treatment for patients with coeliac disease. Limited selection of food options can affect their quality of life and cause problems in acceptance by their peers.

The aim was to examine the subjective quality of life experience in children and adolescents with coeliac disease and to obtain a comprehensive representation of physical and mental impairments and social functioning compared to their healthy peers.

Methods The study was conducted as a cross-sectional study. It included 116 respondents aged 5–18 years with coeliac disease and 116 healthy children of similar age and sex. A Serbian version of Pediatric Quality of Life Inventory (PedsQL) was used to measure the quality of life in children. Descriptive statistics were calculated to analyze all results, while t-test was used to compare them.

Results The mean value of total PedsQL score was lower in the coeliac disease patients (75.89 ± 20.35) than in the controls (86.35 ± 11.13). Additionally, the experimental group reported lower all PedsQL Scale scores than the control group in the domains of psychosocial, school, social, and emotional functioning. However, there was no statistically significant difference on the physical health scale. These results were the same in all age groups among both males and females.

Conclusions The disturbance of health-related quality of life in children and adolescents with coeliac disease is significant and the quality of life is lower if compared to their healthy peers.

Keywords: coeliac disease; children; quality of life

INTRODUCTION

Coeliac disease is a chronic inflammatory disease of multifactorial etiology, which results from a polygenic predisposition and gluten exposure [1]. The only scientifically proven beneficial treatment is strict lifelong adherence to a gluten-free diet [2]. Limited selection of food options, illness-based social inequality if compared to others, illness-induced stress, and the disease treatment can all affect the quality of life. Children, especially if they are ill, can show very intense emotional reactions. This can affect their social functioning because thez might feel shame; children can avoid contacts and having meals with others and, on the other hand, their peers sometimes may have a problem in social acceptance of celiac patients [3, 4].

Health-related quality of life is a subjective feeling of the impact of health on various aspects of life and is focused on children's everyday functioning. However, one of the biggest problems in assessing the quality of life in children is the specificity of their age and whether "their opinion should be taken into account" [5]. Measurement of health and quality of life in children is very complex and associated with various methodological problems. Worldwide previous research indicates that the quality of life of a sick child is significantly influenced by social, psychological, and emotional aspects, apart from the physical ones [6]. Developmental psychologists believe that children aged 5–7 years are able to answer specific questions about their pain, mood and feelings, likes and dislikes, fears induced by certain phenomena, anxiety, and delight, while at the age of nine or ten, they can perform self-assessment and analyze their behaviour [7, 8].

The aim of this paper was to investigate the difference between the subjective experience of the health-related quality of life in children and adolescents with coeliac disease and their

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healthy peers and to obtain a comprehensive picture of their physical and mental impairments and social functioning in comparison to their healthy peers.

METHODS

The study was conducted as a cohort, observational, and cross-sectional study. The study included 116 subjects aged 5–18 years who had been diagnosed with coeliac disease and who complied with a gluten-free diet for at least a year. The control group consisted of 116 healthy respondents who were age and sex matched with the study group. The survey of children with coeliac disease was conducted within regular gastroenterological follow-ups. The selection of respondents for the control group was made using random sampling from the basic set of pre-school, primary, and secondary school children from Belgrade. Children with coeliac disease and comorbidity (Hashimoto thyroiditis, diabetes mellitus, etc.) were excluded from the study.

Pediatric Quality of Life Questionnaire (PedsQL) was the instrument employed for assessing the quality of life (QOL). There were different versions for three age groups (5–7, 8–12, and 13–18 years). Each version had 23 questions sorted into four categories, and answers were graded on a five-point scale. The survey used a variety of Likert-scaled questions: 0 – never, 1 – almost never, 2 – sometimes, 3 – often, and 4 – always. The scales were: Emotional functioning (eight questions), Social functioning (five questions), School functioning (five questions) and Physical functioning (five questions). The questions from the first three scales gave the score of Psychosocial Health, while the last scale was the score of Physical Health. The mean values of results were converted to scale values from 0 to 100, where the higher score indicated better QOL. If more than 50% of the answers were missing, the result was not taken into consideration. PedsQL was culturally adapted and validated in the Serbian language [9, 10].

The questionnaires with brief instructions were given to children and adolescents to fill in, offering the assistance in completing if needed. Parents took an active part in the group of respondents aged 5–7 years. The examination was carried out in the presence of the study authors, nurses and – in the control group – psychologists or pedagogues.

Descriptive statistics were calculated for all questionnaire scores. Independent samples t-test was used to compare PedsQL scores for various groups. The p-value < 0.05 was considered statistically significant. The amount of missing data was 2.6%.

The study was reviewed and approved by the Ethics Committee of the Dr Vukan Čupić Institute of Mother and Child Health in Belgrade and the University Children's Hospital in Belgrade. According to the ethical requirements, all respondents were informed in writing about the study goals and use of the data obtained exclusively for scientific purposes, providing the guarantee of anonymity of all the received data and identity of the respondents.

RESULTS

The basic demographic data of the examined groups has been shown in Table 1. The mean value of PedsQL total score was lower in the coeliac disease group than in the control group. Additionally, the coeliac disease group reported lower all PedsQL Scale scores than the control group. However, there was no statistically significant difference on the Physical health scale (Table 2.).

Table 1. Demographic characteristics of participnts

Parameters	Celiac disease (n = 116)	Control group (n = 116)
Age group, n (%)		
5–7 years	22 (19)	14 (12.1)
8–12 years	44 (37.9)	48 (41.4)
13–18 years	50 (43.1)	54 (46.5)
Sex, n (%)		
male	39 (33.6)	47 (40.5)
female	77 (66.4)	69 (59.5)

Table 2. Children's and healthy peers Pediatric Quality of Life Inventory General Scale Scores

PedsQL	Groups	M	SD	CI 95%		t-test
				LL	UL	(p)
Emotional functioning	Celiac disease	71.29	25.01	66.69	75.89	
	Healthy peers	84.66	15.4	81.82	87.49	**< 0.05**
Social functioning	Celiac disease	81.55	24.02	77.13	85.97	
	Healthy peers	93.97	9.88	92.15	95.78	**< 0.05**
School functioning	Celiac disease	73.81	24.04	69.32	78.29	
	Healthy peers	88.28	13.69	85.76	90.79	**< 0.05**
Physical health	Celiac disease	76.75	25.31	72.1	81.41	
	Healthy peers	81.44	17.13	78.29	84.59	0.100
Psychosocial health	Celiac disease	74.91	21.31	71	78.83	
	Healthy peers	88.97	10.74	86.99	90.94	**< 0.05**
Total	Celiac disease	75.89	20.35	72.14	79.63	
	Healthy peers	86.35	11.13	84.3	88.39	**< 0.05**

M – mean value; SD – standard deviation; CI 95% – confidence interval; LL – lower, UL – upper

Females scored PedsQL higher than males in both groups, with the exception noted in the Emotional functioning scale. Both, males and females from the control group tended to rate the PedsQL scores higher than the coeliac disease group. Among males, differences were statistically significant on four out of six scores with no difference on the Emotional functioning and the Physical health scales. Among females, differences were significant on five out of six scores with no difference on the Physical health scale (Table 3).

The coeliac disease group reported lower all PedsQL Scale scores than the control group across all ages, five of six scales were different. However, there was no difference on the Physical health scale in each age subgroup. In addition, the biggest difference in each domain of quality of life was observed among the youngest age groups (Table 4).

DISCUSSION

Our study included more female participants, which is in line with the results of other surveys. Namely, the authors

Table 3. Children's and healthy peers Pediatric Quality of Life Inventory General Scale Scores according to sex

PedsQL	Groups	Sex	M	SD	t-test	t_{Male}	t_{Female}
					(p)	(p)	(p)
Emotional functioning	Celiac disease	Male	73.08	24.86	0.587		
		Female	70.39	25.21		0.100	**< 0.05**
	Healthy peers	Male	80.9	15.43	0.061		
		Female	86.56	15.14			
Social functioning	Celiac disease	Male	76.03	27.77	**< 0.05**		
		Female	84.35	21.54		**< 0.05**	**< 0.05**
	Healthy peers	Male	93.59	9.1	0.772		
		Female	94.16	10.3			
School functioning	Celiac disease	Male	68.78	25.75	**< 0.05**		
		Female	76.25	22.94		**< 0.05**	**< 0.05**
	Healthy peers	Male	84.74	18.03	**< 0.05**		
		Female	90.06	10.56			
Physical health	Celiac disease	Male	74.48	28.29	**< 0.05**		
		Female	78.61	23.64		0.693	0.065
	Healthy peers	Male	75.24	18.95	**< 0 .05**		
		Female	79.58	15.32			
Psychosocial health	Celiac disease	Male	71.45	23.2	**< 0.05**		
		Female	76.67	20.21		**< 0.05**	**< 0.05**
	Healthy peers	Male	86.41	11.84	**< 0.05**		
		Female	90.26	9.97			
Total	Celiac disease	Male	72.92	21.71	**< 0.05**		
		Female	77.39	19.61		**< 0.05**	**< 0.05**
	Healthy peers	Male	82.53	12.19	**< 0.05**		
		Female	88.28	10.1			

M – mean value; SD – standard deviation

have noticed that female population is more likely to suffer from coeliac disease (1.3–2:1) [1, 11]. In our cohort, nearly 45% of children with coeliac disease belong to the older age group. This is understandable considering the easier cooperation with them, a longer period of treatment in most of them and a better understanding of the disease.

To our knowledge, this is the first study in Serbia to show the differences between the subjective experiences of quality of life dealing with the health of children with coeliac disease in comparison to their healthy peers. While considering individual QOL domains, the lower value can be noticed on five out of six PedsQL scores, with the exception of physical functioning. This should be taken lightly, because, in most cases, the endangering of physical health is usually experienced only through some disabilities. On the other hand, the results of some studies indicate that the timely diagnosis of the disease and gluten-free diet as coeliac treatment do not really affect physical health [11–15].

Dietary restrictions in a gluten-free diet limit various daily social activities including travel and entertainment. They often have to explain why they should not consume gluten-containing food, which often attracts their peer's attention. Teenagers are expected to have more difficulties in adhering to diet due to their rebellious behaviour and the pressure and influence of the society, especially when they have meals at school, visit their friends, go to parties and field trips, etc. There are also difficulties in purchasing gluten-free products; they are often not sufficiently secure and easily accessible, and their price is still a burning issue. All this can be frustrating and bothering for children and can lead to dietary neglect and worsen the disease [14–18].

The emotional functioning of children with coeliac disease is significantly lower in comparison to healthy children. Sick children can show very intense emotional reactions. Similarly, some studies showed that children with coeliac disease had more negative emotions (anger, sorrow, aggression, jealousy) in comparison to their healthy peers [17, 18, 19].

Adaptation to school, completing school obligations, and belonging to various groups are significant indicators of normal development and functioning of children with chronic illness. Because of their strict adherence to dietary regimens and regular medical follow-ups, children do not attend school normally, they need to miss classes, they fail to attend out-of-class activities and lose interaction with their peers. The results of some studies showed that the affected children also express concern about their absence from school, separation from peers, feeling of dissimilarity and they express the increased need for care and assistance from others [17, 19]. Also, there was often the lack of understanding the nature of their illness reflected by some teachers and peers [20].

Observing the sample in our study by sex, we found that girls had higher results than boys, with the exception of emotional functioning. The observation of girls only suggests the conclusion that there were differences between groups in every scale, except in the physical health scale, and the observation of boys showed no differences only

Table 4. Children's and healthy peers Pediatric Quality of Life Inventory General Scale Scores according to age

PedsQL	Groups	Age (years)	M	SD	t_{5-7} (p)	t_{8-12} (p)	t_{13-18} (p)
Emotional functioning	Celiac disease	5–7	59.09	34.9			
		8–12	74.55	23.1			
		13–18	73.8	19.89	**< 0.05**	**< 0.05**	**< 0.05**
	Healthy peers	5–7	81.36	18.85			
		8–12	89.55	13.29			
		13–18	81.8	14.66			
Social functioning	Celiac disease	5–7	74.55	30.04			
		8–12	85.23	19.97			
		13–18	81.4	24.16	**< 0.05**	**< 0.05**	**< 0.05**
	Healthy peers	5–7	90.45	11.74			
		8–12	95.57	6.92			
		13–18	94.1	10.96			
School functioning	Celiac disease	5–7	47.89	29.36			
		8–12	81.93	19.77			
		13–18	76.5	18.27	**< 0.05**	**< 0.05**	**< 0.05**
	Healthy peers	5–7	88.18	16.22			
		8–12	90.91	12.91			
		13–18	86	13.01			
Physical health	Celiac disease	5–7	79.3	32.4			
		8–12	78.34	24.63			
		13–18	77.88	22.66	0.251	0.387	0.390
	Healthy peers	5–7	81.82	10.37			
		8–12	82.46	19.47			
		13–18	80.38	17.54			
Psychosocial health	Celiac disease	5–7	58.33	26.82			
		8–12	80.57	18.74			
		13–18	77.23	17.06	**< 0.05**	**< 0.05**	**< 0.05**
	Healthy peers	5–7	86.67	13.21			
		8–12	92.01	9.47			
		13–18	87.3	10.18			
Total	Celiac disease	5–7	65.29	26.56			
		8–12	79.79	18.62			
		13–18	77.11	17.32	**< 0.05**	**< 0.05**	**< 0.05**
	Healthy peers	5–7	84.98	11.1			
		8–12	88.69	11.52			
		13–18	84.89	10.66			

M – mean value; SD – standard deviation

in emotional functioning and physical health. The results of other researchers are contradictory, some indicated that there is no difference between boys and girls [21, 22], but there are also studies presenting lower quality of life in girls with coeliac disease [23, 24].

Observing coeliac respondents in our study by age, we conclude that they reported a lower level of quality of life in all scores and in all age groups if compared to the control group. The most pronounced deviations were in the youngest age groups. This must be taken with caution, given that in this age parents mostly assisted in giving answers and they do not often have a realistic view when it comes to their children. Surprisingly enough, the greatest difference would be expected to appear between adolescents, considering the peer pressure and busy lifestyle, which contributes to a greater reliance on pre-packaged foods that often contain gluten [4, 12, 14, 18, 25].

The assessment of the health-related quality of life is increasingly recognized as a very important measure of the overall outcome of treatment. Self-reporting on the quality of life allows the examination of factors that affect the illness and treatment from the child's aspect. Dietary changes and the implementation of a gluten-free diet can be difficult, costly, and socially unaccepted due to social constraints, and this creates difficulties in managing the dietary regimen and therefore can exert a negative impact [26–29]. After establishing a definite diagnosis of the disease, and the start of a gluten-free diet rapid recovery takes place, and the quality of their life becomes similar to their healthy peers [12, 30].

CONCLUSION

Compared to healthy children, the quality of life in children with coeliac disease in all age categories is disturbed in every domain where the quality of life can be measured and observed, except physical functioning. Girls with coeliac disease have a better quality of life compared to boys.

For a healthy life with coeliac disease and for successful adaptation and re-socialization of the patients, it is necessary to improve the knowledge about the disease through additional education, to provide the patients with greater choice of gluten-free products, to encourage both patients and their peers to accept the necessary changes and, of course, to provide psychological support.

Conflict of interest: None declared.

REFERENCES

1. Radlović N. Celiac disease. Srp Arh Celok Lek. 2013; 141(1–2):122–6.
2. Pietzak M. Follow-up of patients with celiac disease: Achieving compliance with treatment. Gastroenterology. 2005; 128(4 Suppl):S135–41.
3. Sverker A, Hensing G, Hallert C. Controlled by food-lived experiences of coeliac disease. J Hum Nutr Diet. 2005; 18(3):171–80.
4. Youssef NN, Murphy TG, Langseder AL, Rosh JR. Quality of life for children with functional abdominal pain: a comparison study of patients' and parents' perceptions. Pediatrics. 2006; 117(1):54–9.
5. Beckung E, White-Koning M, Marcelli M, McManus V, Michelsen S, Parkes J, et al. Health status of children with cerebral palsy living in Europe: a multi-centre study. Child Care Health Dev. 2008; 34(6):806–14.
6. Simeoni MC, Auquier P, Antoniotti S, Sapin C, San Marco JL. Validation of a French health-related quality of life instrument for adolescents: the VSP-A. Qual Life Res. 2000; 9(4):393–403.
7. Di Filippo T, Orlando MF, Concialdi G, La Grutta S, Lo Baido R, Epifanio MS, et al. The quality of life in developing age children with celiac disease. Minerva Pediatr. 2013; 65(6):599–608.
8. Tapsas D, Fälth-Magnusson K, Högberg L, Hammersjö JÅ, Hollén E. Swedish children with celiac disease comply well with a gluten-free diet, and most include oats without reporting any adverse effects: a long-term follow-up study. Nutr Res. 2014; 34(5):436–41.
9. Varni JW, Seid MD, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Med Care. 2001; 39(8):800–12.
10. Stevanović D, Lakić A, Damnjanović M. Some psychometric properties of the Pediatric Quality of Life Inventory™ Version 4.0 Generic Core Scales (PedsQL™) in the general Serbian population. Qual Life Res. 2011; 20(6):945–9.
11. Sevinç E, Çetin FH, Coşkun BD. Psychopathology, quality of life, and related factors in children with celiac disease. J Pediatr (Rio J). 2017; 93(3):267–73.
12. Wagner G, Berger G, Sinnreich U, Grylli V, Schober E, Huber WD, et al. Quality of life in adolescents with treated coeliac disease: Influence of compliance and age at diagnosis. J Pediatr Gastroenterol Nutr. 2008; 47(5):555–61.
13. Kolsteren MM, Koopman HM, Schalekamp G, Mearin ML. Health-related quality of life in children with celiac disease. J Pediatr. 2001; 138(4):593–5.
14. Esenyel S, Unal F, Vural P. Depression and anxiety in child and adolescents with follow-up celiac disease and in their families. Turk J Gastroenterol. 2014; 25(4):381–5.
15. Pico M, Spirito MF. Implementation of a health-related quality of life questionnaire for children and adolescents with celiac disease Arch Argent Pediatr. 2014; 112(1):19–25.
16. Zarkadas M. Living with celiac disease and a gluten-free diet: A Canadian perspective. J Hum Nutr Diet. 2013; 26(1):10–23.
17. Ciacci C, Iavarone A, Siniscalchi M, Romano R, De Rosa A. Psychological dimensions of celiac disease: towards an integrated approach. Dig Dis Sci. 2002; 47(9):2082–7.
18. Byström IM, Hollén E, Fälth-Magnusson K, Johansson A. Health-related quality of life in children and adolescents with celiac disease: from the perspectives of children and parents. Gastroenterol Res Pract. 2012; 2012:986475.
19. Loonen HJ, Grootenhuis MA, Last BF, Koopman HM, Derkx HH. Quality of life in paediatric inflammatory bowel disease measured by a generic and a disease specific questionnaire. Acta Paediatr. 2002; 91(3):348–54.
20. Sainsbury K, Mullan B, Sharpe L. Reduced quality of life in coeliac disease is more strongly associated with depression than gastrointestinal symptoms. J Psychosom Res. 2013; 75(2):135–41.
21. Sarkhy A, El Mouzan MI, Saeed E, Alanazi A, Alghamdi S, Anil S, et al. Socioeconomic impacts of gluten-free diet among Saudi children with celiac disease. Pediatr Gastroenterol Hepatol Nutr. 2016; 19(3):162–7.
22. Chauhan JC, Kumar P, Dutta AK, Basu S, Kumar A. Assessment of dietary compliance to gluten-free diet and psychosocial problems in indian children with celiac disease. Indian J Pediatr. 2010; 77(6):649–54.
23. Altobelli E, Paduano R, Gentile T, Caloisi C, Marziliano C, Necozione S, et al. Health-related quality of life in children and adolescents with celiac disease: survey of a population from central Italy. Health Qual Life Outcomes. 2013; 11:204.
24. Haapamaki J, Roine RP, Sintonen H, Kolho KL. Health-related quality of life in paediatric patients with inflammatory bowel disease related to disease activity. J Paediatr Child Health. 2011; 47(11):832–7.
25. Jacobsson LR, Hallert C, Milberg A, Friedrichsen M. Coeliac disease women's experiences in everyday life. J Clin Nurs. 2012; 21(23-24):3442–50.
26. Ciacci C, Iavarone A, Siniscalchi M, Romano R, De Rosa A. Psychological dimensions of celiac disease. Dig Dis Sci. 2002; 47(9):2082–7.
27. Rose C, Howard R. Living with coeliac disease: A grounded theory study. J Hum Nutr Diet. 2014; 27(1):30–40.
28. Black JL, Orfila C. Impact of coeliac disease on dietary habits and quality of life. J Hum Nutr Diet. 2011; 24(6):582–7.
29. Rosen A, Ivarsson A, Nordyke K, Karlsson E, Carlsson A, Danielsson L, et al. Balancing health benefits and social sacrifices: a qualitative study of how screening-detected celiac disease impacts adolescents' quality of life. BMC Pediatr. 2011; 11:32.
30. Barratt SM, Leeds JS, Sanders DS. Quality of life in Coeliac Disease is determined by perceived degree of difficulty adhering to a gluten-free diet, not the level of dietary adherence ultimately achieved. J Gastrointestin Liver Dis. 2011; 20(3):241–5.

Процена квалитета живота и менталног здравља деце и адолесцената са целијакијом и њихових здравих вршњака

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САЖЕТАК

Увод/Циљ Строго контролисана и доживотна безглутенска дијета је лек за болеснике са целијакијом. Ограничен избор хране им ствара проблеме у прихватању од стране вршњака и може имати утицај на квалитет живота и психичко стање. Циљ рада је да се испита субјективан доживљај квалитета живота деце и адолесцента са целијакијом и добије целовита слика о нарушености њиховог физичког и психичког здравља и социјалног функционисања у односу на здраве вршњаке.

Методе Истраживање је спроведено по типу студије пресека. Студијом је обухваћено 116 испитаника узраста 5–18 година са целијакијом и 116 здраве деце сличног узраста и пола. Српска верзија Педијатријског упитника о квалитету живота (*PedsQL*) коришћена је као инструмент мерења квалитета живота деце. Дескриптивни подаци израчунати су за све резултате, док је *t*-тест коришћен да их упореди.

Резултати Средња вредност укупног броја бодова у *PedsQL* била је нижа у групи деце и адолесцената са целијакијом (75,89 ± 20,35) него у контролној групи (86,35 ± 11,13). Експериментална група је такође имала ниже резултате *PedsQL* у односу на контролну групу у доменима психосоцијалног, школског, социјалног и емоционалног функционисања. Није нађена статистички значајна разлика у домену показатеља физичког здравља. Ови резултати су били исти код дечака и девојчица свих узраста.

Закључак Квалитет живота деце и адолесцената са целијакијом знатно је нарушен у поређењу са здравим вршњацима.

Кључне речи: целијакија; деца; квалитет живота

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Laparoscopic technique as a method of choice in the treatment of non-parasitic splenic cysts

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SUMMARY

Introduction/Objective Splenic cysts are a rare clinical entity. In their origin, they may be parasitic or non-parasitic. They are classified as either primary (true) or secondary cysts (pseudocysts), depending on the presence or absence of an epithelial lining of the lumen.

Methods Using a retrospective case study, we included 29 patients undergoing laparoscopic surgery due to splenic cysts. The patients were treated within the 2007–2017 period at the Clinic for Digestive Surgery, the Clinical Center of Serbia. We analyzed pre-operative, intra-operative and post-operative characteristics of laparoscopic technique in treating non-parasitic splenic cysts.

Results The group of 29 patients treated with laparoscopic technique surgery due to the previously diagnosed splenic cyst, consisted of 12 (41.4%) men and 17 (58.6%) women. The average age of patients undergoing surgery was 38.86 ± 10.4 years (22–62). Based on the histopathological findings, there were eight epithelial cysts and 21 pseudocysts of the spleen. The maximal diameter of the splenic cyst, measured pre-operatively, was 10.09 ± 2.51 cm on average. The average operative time was 35 minutes, and the intra-operative bleeding amount was 11.48 ± 3.78 ml. None of the intra-operative complications, injury to the spleen or other organs of the abdomen, conversions to open surgery or reintervention were recorded.

Conclusion Laparoscopic fenestration with excision of the part of the spleen cyst wall (marsupialization) represents an effective and definitive treatment for this disease. It is an alternative to splenectomy, along with other well-known benefits ensured by the spleen preservation. Low probability of occurrence of intra-operative and post-operative complications, as well as minimal trauma of tissue, results in a shorter postoperative stay, rapid recovery, and better quality of life.

Keywords: cyst; spleen; laparoscopy; laparoscopic technique

INTRODUCTION

Splenic cysts are a rare disease. In their origin, they can be parasitic and non-parasitic. Echinococcal splenic cyst is the most common type of parasitic ones [1]. Depending on the presence of epithelium that covers the lumen of the cyst, cysts are divided into primary (true) and secondary (pseudocysts). The primary ones are epithelial (epidermoid, dermoid, mesothelial) or endothelial (hemangioma, lymphangioma) [2]. Primary cysts are most commonly found in children and younger persons, comprising about 25% of all non-parasitic cysts. In most cases, they are asymptomatic [3]. Secondary cysts are usually the result of trauma [1]. Common physical symptoms are pain or discomfort in the left upper abdominal quadrant, nausea and vomiting resulting from the compressive effect on the stomach. Larger sized splenic cysts can be presented as a palpable mass in the upper part of the abdomen [4].

Various treatment options are used to treat splenic cysts. A conservative treatment, followed through regular diagnostic examinations (abdomen ultrasound, computerized tomography, magnetic resonance), is generally applied when the cyst is less than 5 cm in diameter and not a symptomatic one [5]. When the diameter of the cyst exceeds 5 cm or in case of any associated symptomatology, surgical treatment is required due to possible complications such as bleeding, cyst rupture and abscess formation [6]. Other treatment modalities include splenectomy, partial splenectomy, puncture-aspiration-injection-reaspiration (PAIR) technique, partial excision, and marsupialization of the splenic cysts. Spleen preservation should be considered as the most important factor in selecting a surgical procedure [7].

The benefit of the splenic preservation during surgery is in the fact that the splenectomized patients are at high risk of post-splenectomy sepsis. It can occur five years after the surgery, and in very rare cases a decade after the surgery. This indicates a long-term increased infection risk at splenectomized patients [8].

The objectives of this study are the analysis of the pre-operative characteristics of the patients treated with laparoscopic surgery, as well as the intra-operative parameters, bleeding, and surgery duration. Post-operative parameters, the duration of post-operative hospital stay, the timing of drain removal, as well as the evaluation

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of the effectiveness of laparoscopic treatment have also been within the scope of this analysis.

METHODS

Using a retrospective case study, we included 29 patients undergoing laparoscopic surgery due to splenic cysts. The patients were treated within the 2007–2017 period at the Clinic for Digestive Surgery of the Clinical Center of Serbia, Belgrade, in accord with standards of the institutional Committee on Ethics. As a part of the pre-operative investigation, the patients had undergone diagnostic imaging (ultrasound examination, computerized tomography or magnetic resonance imaging), which provided data regarding the size of the cyst. Serological analyses of parasitic diseases and tumor marker tests had been performed before surgery in the pre-operative assessment. Tumor markers Ca 19.9 and CEA had normal values in all the patients. The study excluded patients with proven parasitic splenic disorder, patients in whom, based on imaging findings (e.g. cystic change involves the splenic hilum, large cysts with minimal residual normal splenic tissue, etc.), decision to undergo total splenectomy had been made, as well as patients with contraindications for laparoscopic surgery due to comorbidity. Most of our patients were asymptomatic. A small group of patients had unspecific complaints in the form of dull pain in the upper abdomen or feeling of abdominal discomfort. We had no pregnant women in our study.

The parameters we monitored in this study were divided into the following three groups: 1. Pre-operative, including: sex, age, body mass index (BMI), size of splenic cysts; 2. Intra-operative, including the duration of the operation, intraoperative blood loss, intraoperative complications (conversions, instrumental injuries of the spleen and other organs) 3; Post-operative, including: the duration of postoperative stay, time of drain removal, post-operative complications, reintervention, histopathological findings based on which the cysts have been classified, as well as the average duration of postoperative monitoring of the treated patients.

In all the treated patients, laparoscopic fenestration and wall cyst excision was done along with the spleen preservation. Laparoscopic fenestration with excision of the cyst wall is performed under general endotracheal anesthesia. The patients were placed in the right lateral position, i.e. so-called hanging spleen technique position, due to its advantages, which so far have been proven on numerous occasions [9]. Cyst fenestration and the aspiration of its content is performed by laparoscopic harmonic scalpel (Ultracision®, Ethicon Inc., Somerville, NJ, USA) (Figure 1). The cyst wall is excised to the maximum possible extent. A part of a large omentum is placed in the cyst cavity after rinsing, aspiration, and proven homeostasis. At the end of the surgery, the drain is placed in the left subphrenic space.

The Clavien–Dindo Scale was used to define the ranking of postoperative complications. The first postoperative examination took place three months after the operation and included an abdominal ultrasound. The second one, a year after, included CT scan. Every six months from then, patients were checked-up by abdominal ultrasound. Five years after the operation, an abdominal ultrasound examination was performed once a year.

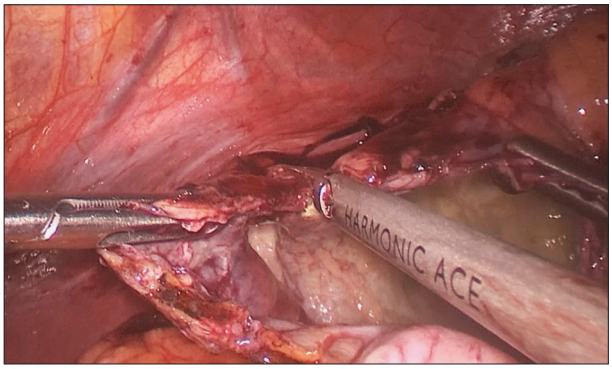

Figure 1. Intraoperative photo – cyst wall excision

RESULTS

The group of 29 patients treated with laparoscopic technique due to previously diagnosed splenic cyst consisted of 12 (41.4%) men and 17 (58.6%) women. The average age of patients was 38.86 ± 10.40 years (22–62) and the average weight expressed in BMI was 23.03 ± 2.65. The maximal diameter of the splenic cyst, measured pre-operatively, was 10.09 ± 2.51 cm on average (Table 1).

Abdominal drain was removed on the first postoperative day, while the average postoperative stay was two days (1–3). The average operative time was 35 minutes and intraoperative bleeding volume was 11.48 ± 3.78 ml (Table 2). Based on the histopathological findings, there were eight epithelial cysts and 21 pseudocysts of the spleen. Postoperative complications were classified based on the Dindo–Clavien Scale (Table 3). None of intraoperative complications, injury to the spleen or other organs of the abdomen, conversions to open surgery or reintervention were recorded. The average period of postoperative follow-up was four years (1–10).

DISCUSSION

In 1985, Salky et al. [6] initially reported laparoscopic surgery as a treatment option for splenic cyst, with a good outcome and without recurrence of the disease after eight months. In their series of 32 cases, Robertson et al. [10] found that the recidivism rate after the laparoscopic operation was 22%, but a new surgical intervention was required in only 3% of the patients. In order to reduce the risk of recurrence of the disease, it is considered necessary to remove as much of the wall of the cyst as possible [2, 11, 12].

The modalities of the surgical treatment of splenic cysts are different. They vary from the application of minimally invasive surgical procedures such as cyst fenestration, cyst marsupialization, PAIR, partial splenectomy, and total

Table 1. Preoperative patients' characteristics

Patients' characteristics	Male	Female	Description
Number of patients	12 (41.4)	17 (58.6)	29 (Summary)
Age	40.3	37.8	38.86 ± 10.40 ($\bar{x}$ ± sd)
BMI	22.5	23.4	23.03 ± 2.65 ($\bar{x}$ ± sd)
Cyst size (cm)	10.4	9.9	10.09 ± 2.51 ($\bar{x}$ ± sd)

BMI – body mass index

Table 2. Surgery data

Parameter	Med. (min.–max.) or ($\bar{x}$ ± sd)
Surgery time (minutes)	35 (28–65)
Intraoperative bleeding (ml)	11.48 ± 3.78
Postoperative stay (days)	3 (2–4)
Drainage (days)	2 (1–3)

Table 3. Postoperative complications according to Dindo–Clavien Scale

DC class	Patients n (%)
I	5 (17.2)
II	3 (10.3)
III	0
IV	0
V	0

DC – Dindo–Clavien

splenectomy [7, 13, 14]. Some studies have proven that laparoscopic fenestration in the treatment of non-parasitic splenic cyst is an acceptable treatment method, with an acceptable rate of disease recurrence. The accent is on the need for resecting as much of the cyst wall as possible, in order to prevent recurrence [15, 16]. In our series, a laparoscopic fenestration including wall excision of a splenic cyst was performed in all patients. PAIR technique is applicable in non-parasitic splenic cysts, but carries a higher risk of recurrence, up to 29% in some series [17]. This technique is justified in patients with severe comorbidities and contraindications for operative treatment, as well as in patients who do not consent to surgical treatment. Laparoscopic partial splenectomy can be applied in the treatment of non-parasitic splenic cysts with a low expectancy of recurrence. This technique is limited by the size and position of the cyst in regard to the vascular elements [5].

Spleen is an organ that has an important role in the human immune system. It also plays a significant role in filtering blood, removing old and dead erythrocytes and platelets. Considering all known modalities for treating non-parasitic splenic cysts, preservation of the spleen offers benefit, especially in the younger population [18]. The method of choice for the treatment of benign splenic disorders, particularly in case of simple cysts, is the laparoscopic cyst marsupialization. It can be safely performed provided that the surgeon and the surgical team have the necessary experience and skills in laparoscopy. The minimally invasive surgery enables rapid recovery, without complications usually associated with large laparotomy incisions [19].

The spleen has an important role in clearing bacteria from the circulation. Asplenic individuals are at increased risk of postsplenectomy sepsis (PSS). This fulminant and rapidly fatal illness complicates bacteremic infections and can be seriously threatened in splenectomized patients. The incidence of this syndrome is the highest in children who undergo splenectomy in infancy [20].

Davidson and Wall [21] reported that PSS carries a rather high mortality rate of 50–70%. The highest risk of PSS occurs during the first few years following splenectomy but has been documented as late as 40 years after splenectomy [8]. If surgical preservation of the spleen is not possible, it is recommended to perform pre-operative or post-operative vaccination of the patients within two weeks after splenectomy [9].

The results of our study show that our patients did not have either intra-operative complications, or conversions, but only minimal intraoperative blood loss. In the results published by Cai et al. [16], it is stated that the period of post-surgery hospital stay varied 24–48 hours. The average hospital stay in our series was 48 hours, but some patients were discharged after the first postoperative day.

Postoperative complications were ranked according to the Dindo–Clavien Scale. We had five patients who required additional therapy in the form of analgesics and antiemetics, and only three patients to whom we prescribed additional antibiotic therapy. We did not experience any other complications.

Cuervo and Buela [22] recommended reviewing computerized tomography once a year. Patients had been followed by periodic abdominal ultrasound and computerized tomography scan once a year. The average follow-up period in our study was four years. No case of the disease recurrence was recorded.

CONCLUSION

Laparoscopic fenestration with excision of the part of the spleen cyst wall (marsupialization) represents an effective and definitive treatment for this disease. It is an alternative to splenectomy, along with other well-known benefits ensured by the spleen preservation. The method implies a safe treatment with minimal bleeding. Low probability of occurrence of intra-operative and post-operative complications, as well as minimal trauma of tissue, results in a shorter post-operative stay, rapid recovery and better quality of life.

Conflict of interest: None declared.

REFERENCES

1. Adrian PE, Eduardo TM. Spleen. In: Brunicardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, editors. Schwartz's principles of surgery. Tenth edition. ed. New York: McGraw-Hill Education; 2015. p. xviii, 2069 pages.
2. Shabtaie SA, Hogan AR, Slidell MB. Splenic Cysts. Pediatric Annals. 2016; 45(7):e251–e6.
3. Daniel AA. Spleen. In: Goldblum JR, Lamps LW, McKenney JK, Myers JL, Ackerman LV, Rosai J, editors. Rosai and Ackerman's surgical pathology. 11th edition ed. Philadelphia: Elsevier; 2018. p. 2 volumes.
4. Mirilas P, Mentessidou A, Skandalakis JE. Splenic cysts: are there so many types? J Am Coll Surg. 2007; 204(3):459–65.
5. Manciu S, Tudor S, Vasilescu C. Splenic Cysts: A Strong Indication for a Minimally Invasive Partial Splenectomy. Could the Splenic Hilar Vasculature Type Hold a Defining Role? World J Surg. 2018; 42(11):3543–50.
6. Salky B, Zimmerman M, Bauer J, Gelernt I, Kreel I. Splenic cyst – definitive treatment by laparoscopy. Gastrointest Endosc. 1985; 31(3):213–5.
7. Vijayaraghavan R, Chandrashekar R, Aithal S, Rashmi MV, Belagavi CS. Mesothelial cyst of the spleen in an adult: a case report. BMJ Case Rep. 2010; 2010.
8. Fernández-López AJ, Candel-Arenas M, González-Valverde FM, Luján-Martínez D, Medina-Manuel E, Albarracín Marín-Blázquez A. Escisión laparoscópica en quiste esplénico sintomático. Cirugía y Cirujanos. 2017; 85:26–9.
9. Buzele R, Barbier L, Sauvanet A, Fantin B. Medical complications following splenectomy. J Visc Surg. 2016; 153(4):277–86.
10. Robertson FM, Doski JJ, Cofer BR, Kidd JN. Laparoscopic Splenic Cystectomy: A Rational Approach. Pediatric Endosurgery & Innovative Techniques. 2004; 8(4):321–6.
11. Conlon KC, Geraghty M, Khan IZ. Large primary splenic cyst: A laparoscopic technique. Journal of Minimal Access Surgery. 2009; 5(1):14.
12. Al Khafaji B, Younis MU. Laparoscopic splenic cyst fenestration-a viable spleen preserving option. J Surg Case Rep. 2017; 2017(8):rjx154.
13. Quesada R, Poves I, Iglesias M, Berjano E, Grande L, Burdio F. Laparoscopic partial splenectomy for giant cyst using a radiofrequency-assisted device: a case report. Surg Case Rep. 2016; 2(1):82.
14. Putnik S, Ilic M. Partial resection of the splenic cyst using radiofrequency ablation system. Srp Arh Celok Lek. 2017; 145(5–6):301–3.
15. Farhangi B, Farhangi A, Firouzjahi A, Jahed B. Huge epithelial nonparasitic splenic cyst: A case report and a review of treatment methods. Caspian J Intern Med. 2016; 7(2):146–9.
16. Cai H, An Y, Wu D, Chen X, Zhang Y, Zhu F, et al. Laparoscopic Partial Splenectomy: A Preferred Method for Select Patients. J Laparoendosc Adv Surg Tech A. 2016; 26(12):1010–4.
17. Akhan O, Dagoglu-Kartal MG, Ciftci T, Ozer C, Erbahceci A, Akinci D. Percutaneous Treatment of Non-parasitic Splenic Cysts: Long-Term Results for Single- Versus Multiple-Session Treatment. Cardiovasc Intervent Radiol. 2017; 40(9):1421–30.
18. Hassoun J, Ortega G, Burkhalter LS, Josephs S, Qureshi FG. Management of nonparasitic splenic cysts in children. J Surg Res. 2018; 223:142–8.
19. Kenney CD, Hoeger YE, Yetasook AK, Linn JG, Denham EW, Carbray J, et al. Management of non-parasitic splenic cysts: does size really matter? J Gastrointest Surg. 2014; 18(9):1658–63.
20. Theilacker C, Ludewig K, Serr A, Schimpf J, Held J, Bogelein M, et al. Overwhelming Postsplenectomy Infection: A Prospective Multicenter Cohort Study. Clin Infect Dis. 2016; 62(7):871–8.
21. Davidson RN, Wall RA. Prevention and management of infections in patients without a spleen. Clin Microbiol Infect. 2001; 7(12):657–60.
22. Cuervo JL, Buela E. [Splenic epidermoid cyst: laparoscopic partial decapsulation]. Cir Pediatr. 2007; 20(1):63–7.

Лапароскопска техника као метод избора у третману непаразитних циста слезине

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САЖЕТАК

Увод/Циљ Цисте слезине представљају редак клинички ентитет. Према њиховом пореклу могу бити паразитне и непаразитне. У зависности од присуства или одсуства епителијалног омотача, цисте слезине се деле на примарне (праве) и секундарне (псеудоцисте).

Методе Студија је рађена као ретроспективна студија серије случајева у коју је укључено 29 болесника који су били подвргнути лапароскопском хируршком третману због циста слезине у периоду 2007–2017. године на Клиници за дигестивну хирургију Клиничког центра Србије. Анализиране су преоперативне, интраоперативне и постоперативне карактеристике болесника код којих је била примењена лапароскопска хируршка техника у третману непаразитних циста слезине.

Резултати У групи од 29 болесника оперисаних лапароскопском техником код којих је преоперативно дијагностиковано постојање непаразитних цисти слезине било је 12 (41,4%) мушкараца и 17 (58,6%) жена. Просечна старост оперисаних болесника износила је 38,86 ± 10,40 година (22–62). Хистопатолошким прегледом верификовано је осам епителијалних циста и 21 псеудоциста слезине. Просечна димензија циста слезине која је мерена преоперативно износила је 10,09 ± 2,51 *cm*. Просечно време трајања операције било је 35 минута. Интраоперативни губитак крви износио је просечно 11,48 ± 3,78 *ml*. Није забележена ниједна интраоперативна компликација у смислу неконтролисаног интраоперативног крварења, повреде слезине или суседних органа.

Закључак Лапароскопска операција са одстрањивањем дела зида цисте слезине представља ефикасан третман овог обољења. Метода представља безбедан третман са минималним крварењем, уз малу вероватноћу појаве интраоперативних и постоперативних компликација.

Кључне речи: циста; слезина; лапароскопија; лапароскопска техника

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The influence of endothelial hyperplasia on pseudoprogression development in patients with glioblastoma

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SUMMARY

Introduction/Objective Pseudoprogression represents an enlarging contrast-enhancing lesion that occurs after chemoradiation and stabilizes with time without any changes in the therapeutic procedure. This phenomenon is highly significant, because it can have influence on further therapeutic procedures; however, precise criteria for pseudoprogression diagnosis have not yet been defined. The main goal of this study is to examine the endothelial hyperplasia influence on pseudoprogression.

Methods We analyzed a group of 106 patients with glioblastoma who had undergone surgical treatment from 2010–2012, at the Clinic of Neurosurgery, Clinical Center of Serbia, who received Stupp protocol. Pre- and post-treatment imaging was evaluated using RANO criteria. Lesions that improved or stabilized were defined as pseudoprogression, and lesions that progressed were defined as true progression. Endothelial hyperplasia was identified based on the hematoxylin and eosin pathohistological examination.

Results Thirty-two (30.2%) of the patients were diagnosed with pseudoprogression. Endothelial hyperplasia was observed in 51 (48.1%) of glioblastoma tissue samples, and 28 (87.5%) of all the patients with pseudoprogression were found to have endothelial hyperplasia. The group of 51 (68.9%) patients without pseudoprogression did not show the presence of endothelial hyperplasia. Statistical analysis showed significantly higher incidence of pseudoprogression in patients with endothelial hyperplasia. ($\chi^2 = 26.269$, $p < 0.01$)

Conclusion Taking into account that there are no precise diagnostic methods that could determine the presence of endothelial hyperplasia with certainty, it could be an indicator, as a pathohistological entity, of a higher likelihood of pseudoprogression, which could be used in everyday clinical practice. In order to reach definite conclusions, we believe it is necessary to conduct prospective controlled studies with larger sample sizes.

Keywords: glioblastoma; endothelial hyperplasia; pseudoprogression

INTRODUCTION

Pseudoprogression represents new or enlarging contrast enhancing lesion after chemotherapy within the radiation field that stabilizes with time without any changes in the therapeutic procedure [1]. This phenomenon is highly significant, because it mimics true tumor progression and, if it is misinterpreted as such, it can have a negative influence on the further therapeutic procedures. Pseudoprogression is commonly seen in asymptomatic patients. However, some patients present with clinical deterioration. These complications can include worsening of pre-existing symptoms, transient cognitive decline, subacute rhombencephalitis, or somnolence syndrome [2].

The pathophysiological basis of pseudoprogression remains poorly understood. There are certain indications that it is part of the spectrum of radiation-induced changes ranging from subacute radiation-induced changes to late radiation necrosis [3]. It is assumed that there are two components of pseudoprogression: vascular injuries and treatment-related cell toxicity [4]. It is thought that transient breakdown of the blood brain barrier can cause the edema and contrast enhancement seen on the magnetic resonance imaging (MRI) [5]. The cells most sensitive to radiation are oligodendrocytes, endothelial cells, and neural precursors. Cellular damage can lead to cell death including p53 and p53-independent mechanisms of apoptosis.

Pathohistological examination of patients operated because of pseudoprogression showed distinct characteristics. On the microscopic level, tumor recurrence is usually characterized by microvascular proliferation and highly cellular tumor tissue. This is contradictory to the histological characteristics of the contrast enhancing tissue of pseudoprogression that usually has a low cellularity. Typically, pleomorphic tumor cells can also be found in these lesions with the low mitotic index. In addition, elements of coagulative necrosis can be found, and it appears eosinophilic on light microscopy. Hyalinization of the wall of blood vessels and fibrinoid necrosis is frequent. Telangiectatic blood vessels may be seen, though they are less specific. Fibrillary

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and gemistocytic astrocytes may be observed as well. Scattered pleomorphic astrocytes are mostly associated with the tumor exposed to radiation and are often present in the tissue obtained from pseudoprogression [6].

The accurate diagnosis of pseudoprogression from the true tumor progression is of great significance in planning of further treatment [7]. Modern techniques and MR sequences are developed with the goal of differentiating pseudoprogression and true tumor progression. Diffusion-Weighted Imaging, Diffusion-Tensor Imaging, Perfusion-Weighted Imaging and MR spectroscopy can be helpful, but are not conclusive. Positron Emission Tomography combined with other specific biomarkers is also used along with MRI. However, neither one of the abovementioned methods can be used for diagnosing any pseudoprogression-related changes with certainty.

In this paper, we have examined if endothelial hyperplasia, being one of pathohistological features of glioblastoma, affects the development of pseudoprogression in patients with glioblastoma.

METHODS

We used a retrospective analysis of prospectively collected data in order to analyze the patients with glioblastoma who had undergone surgical treatment during the three-year period (2010–2012), at the Clinic of Neurosurgery, Clinical Center, Serbia and who received Stupp protocol after the surgery. The patients were monitored by a series of MRI scans. While making the final diagnosis, we used the RANO criteria, taking into consideration the fact, established after the numerous literature data, that pseudoprogression may even occur 12 weeks after the chemoradiation has been completed.

Statistical methods

IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for all calculations in the study. Specific measures of central tendency and variability measures were found for continual variability whereas the frequency of the separate categories was specified for the categorical variables. A chi-square test was used for examining the factors that have an influence on pseudoprogression development. The maximum level of acceptability of the null hypothesis probability used in our study is 0.05. The conducted study was approved by the Ethics Committee of the Clinical Centre of Serbia.

RESULTS

A total of 106 patients with glioblastoma who underwent surgery in the period from January 1, 2010 to December 31, 2012 and who underwent Stupp protocol were included in this study. Thirty-two (30.2%) patients were diagnosed with pseudoprogression, whereas 74 (69.8%) of the patients showed no signs of pseudoprogression. On average, pseudoprogression was observed after 4.64 months.

In the group of the patients with pseudoprogression, 59.4% of them were men and 40.6% were women, whereas in the group of the patients with no pseudoprogression, 54.1% of them were men and 45.9% were women. In the group of the patients with pseudoprogression, the average age of patients was 53.03 ± 10.14 years, while in the group of the patients with no signs of pseudoprogression the average age was 53.99 ± 12.53 years. The highest number of patients with developed pseudoprogression was found in the age group ranging from 51–60 years (53.1%), whereas the least number was found in the age group ranging from 71–80 years (3.1%). The greatest number of patients had a radical operation: 74 (70.2%),and out of them 19 patients (17.2%) had subtotal resection, 12 (11.5%) patients had the tumor reduction, whereas there was only one biopsy and that patient was excluded from further analysis (1.1%). Pseudoprogression occurred in 15 (46.7%) of the patients who had received the radical surgery, it occurred in 11 cases (33.3%) of the patients who had had subtotal tumor resection, whereas six (20%) of the patients with pseudoprogression had the tumor reduction. The influence of a degree of tumor resection on the incidence of pseudoprogression was not significant ($\chi^2 = 5.493$, $p = 0.139$).

Endothelial hyperplasia was observed in 51 (48.1%) of glioblastoma tissue samples. Twenty-eight (87.5%) of all patients with pseudoprogression were found to have endothelial hyperplasia, whereas four patients (12.5%) were without it. Fifty-one (68.9%) patients who were not diagnosed with pseudoprogression did not show the presence of endothelial hyperplasia either, whereas it was shown that 23 (31.1%) of the patients who were not diagnosed with pseudoprogression had endothelial hyperplasia. In the group of patients who developed pseudoprogression, the number of patients who had endothelial hyperplasia described in their pathohistological findings was statistically significantly higher, whereas there were more patients who were not diagnosed with pseudoprogression and who did not have endothelial hyperplasia. ($\chi^2 = 26.269$, $p < 0.01$)

DISCUSSION

Pseudoprogression occurred in 32 patients (30.2%). When we compared our results with the literature data, we found the heterogeneity in the data related to the incidence of pseudoprogression [8, 9, 10]. The study undertaken in 2017, which included the papers written in the period from 2005 to October 8, 2014 due to the meta-analysis, published that pseudoprogression was present in 36% of patients [11]. The reason for this may be found in various criteria for its defining along with the fact that the results of a specific number of papers were based on small sample sizes. In our study, we used the RANO criteria for defining the pseudoprogression (Figure1). However, we carefully approached one specific piece of information related to the incidence of pseudoprogression occurring even within 12 weeks after completing chemo-irradiation, because of various literature data claiming that pseudoprogression can be observed much later and that it can be observed

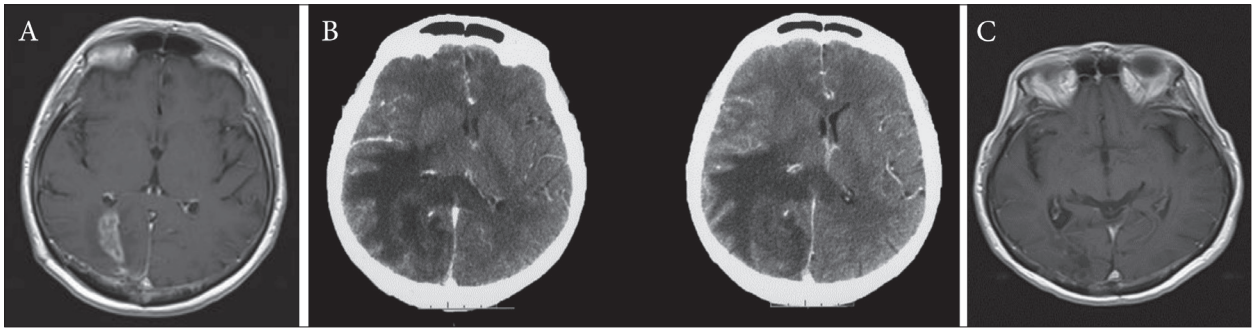

Figure 1. A – The first endocranial magnetic resonance imaging after treatment, radiological progression without clinical deterioration; B – endocranial computed tomography scan after two months, extensive edema followed by clinical deterioration; C – complete regression after corticosteroid therapy and continuing with chemotherapy

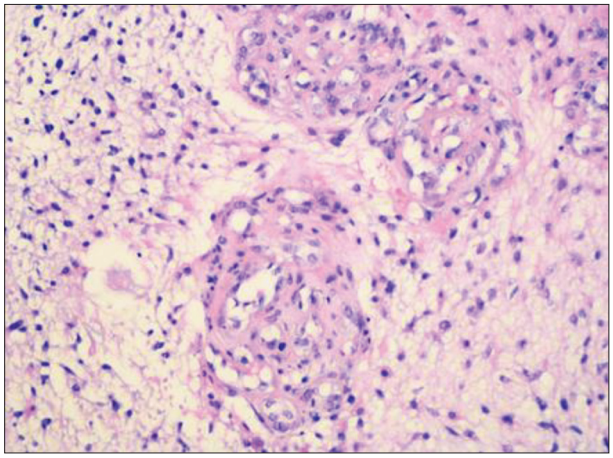

Figure 2. Endothelial hyperplasia, formation of glomeruloid structures

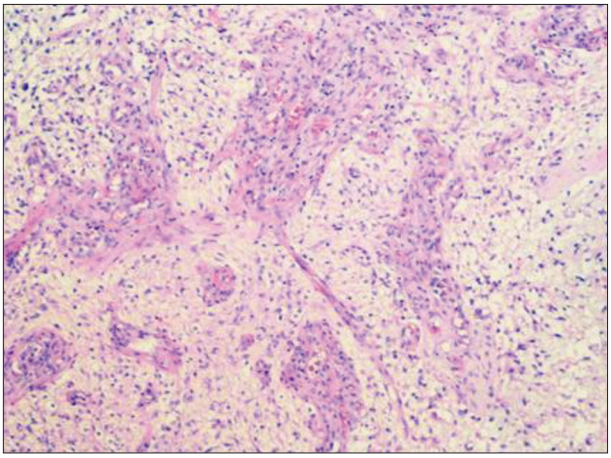

Figure 3. Endothelial hyperplasia and necrosis

even later than 40 weeks and more [12, 8]. Our research demonstrated that pseudoprogression occurred on an average of 4.64 months. The highest number of patients with pseudoprogression was found in the age group ranging from 51–60 years, and then in the age group ranging from 61–70 years. In a study published by Chu et al. [13], the average age of the patients with pseudoprogression was 46.66 ± 15.34 years, which is similar to the results obtained in our study. Endothelial hyperplasia, commonly characterized by the formation of glomeruloid structures, represents one of the main characteristics of glioblastoma (Figure 2). It is usually located in the vicinity of necrosis and appears directionally oriented to it (Figure 3). After the analysis of all the scientific papers published so far, we have not been able to find the results dealing with the influence of endothelial hyperplasia on pseudoprogression development. The results obtained in our study demonstrated that pathohistological characteristics such as endothelial hyperplasia, are statistically significantly higher in the patients developing pseudoprogression. This phenomenon could be primarily explained in terms of disturbing the integrity and normal functions of the blood-brain barrier (BBB). Namely, one of the pathophysiological mechanisms explaining the incidence of pseudoprogression is increased permeability of the BBB [5]. The BBB is an extremely important structure that maintains the balance of the central nervous system (CNS) microenvironment and maintains the normal functioning of the brain. The BBB is constituted of endothelial cells, astrocytes, peripheral cells, macrophage, fibroblasts, neuronal cells, basement membranes, microglia, and other cell types. There are many transporters on the BBB, including P-glycoprotein (P-gp). Astrocytes are involved in nerve signal transmission, nutrient transport, maintaining the balance of brain microenvironment and extracellular matrix ion balance buffering. Peripheral cells are multifunctional cells, with immune function in the CNS neurovascular unit. Peripheral cells surround the endothelial cells and play an important role in the BBB microenvironment and in maintaining the BBB function by secreting growth factors and extracellular matrix. Microglia are a kind of long-standing immune cell in the human brain. They can stimulate the opening of BBB, leukocyte extravasation, and angiogenesis. Fibroblasts, when co-cultured with glioblastoma cells, can induce production and activation of matrix metalloproteinase MMP2, and its activators membrane type 1 metalloproteinase (MT1-MMP) and MT2-MMP, which affect the growth progression of gliomas. Other cells, like endothelial cells, in the BBB microenvironment maintain the normal function and integrity of the BBB by forming tight junctions that limit transcytosis. CNS neurons bind chemicals and convey electrical signals. They can regulate the ionic microenvironment of the synaptic and axonal regions of the nerve cell, which are essential to the nerve signal transduction. The basement membrane is attached as a support tissue to the neurovascular unit cells. The

endothelial cell is the most important structural component of the BBB. Changes in the phosphorylation state of the tight junction protein (ZO-1 or occluding) are critical to the control of BBB vascular permeability. In areas of tumor environment, the endothelial cells connection is very loose and almost lacks integrity [14]. Endothelial hyperplasia is a frequent finding in glioblastoma and it is connected with an increase in nonselective transport through the BBB [15]. The major changes reflect in an increased number of endothelial cells, endothelial hyperplasia leading to function loss and volume reduction in the endothelial cells, cell form changes, tight junction damage, an increased number of vesicles, caveolae and fenestrations, the basement membrane thickening, perivascular space expansion, and the necrosis of capillary endothelial cells [16–19]. All the above-mentioned changes lead to BBB degradation. A significant degradation of the integrity and an increase in the BBB permeability in patients with endothelial hyperplasia may be the cause of contrast leaking the blood vessels, which may eventually induce radiology changes described under the notion of pseudoprogression.

CONCLUSION

Pseudoprogression is a phenomenon of great clinical significance. Distinguishing pseudoprogression from true tumor progression has significant influence on further treatment of patients with glioblastoma. Considering the fact that no accurate diagnostic method has been found so far due to which it would be possible to undoubtedly confirm the presence of pseudoprogression, the presence of endothelial hyperplasia as a pathohistological entity could be an indicator of a higher likelihood of pseudoprogression, which could be used in everyday clinical practice. Nevertheless, in order to reach definite conclusions, we believe it is necessary to conduct prospective controlled studies with larger sample sizes.

NOTE

This paper is based on Dr. Marko Petrović's PhD thesis.

Conflict of interest: None declared.

REFERENCES

1. Gahramanov S, Raslan AM, Muldoon LL, Hamilton BE, Rooney WD, Varallyay, et al. Potential for differentiation of pseudoprogression from true tumor progression with dynamic susceptibility-weighted contrast-enhanced magnetic resonance imaging using ferumoxytol versus gadoteridol: a pilot study. Int J Radiat Oncol Biol Phys. 2011; 79(2):514–23.
2. Fink J, Born D, Chamberlain MC. Radiation necrosis: Relevance with respect to treatment of primary and secondary brain tumors. Curr Neurol Neurosci Rep. 2012; 12(3):276–85.
3. Huang RY, Neagu MR, Reardon DA, Wen PY. Pitfalls in the neuroimaging of glioblastoma in the era of antiangiogenic and immuno/targeted therapy - detecting illusive disease, defining response. Front Neurol. 2015; 6:1–16.
4. Herbert B. Newton. Handbook of Neuro-oncology Neuroimaging. 2nd ed. USA: Academic Press; 2016.
5. Yaman E, Buyukberber S, Benekli M, Oner Y, Coskun U, Akmansu M, et al. Radiation induced early necrosis in patients with malignant gliomas receiving temozolomide. Clin Neurol Neurosurg. 2010; 112(8):662–7.
6. Woodworth GF, Garzon-Muvdi T, Ye X, Blakeley JO, Weingart JD, Burger PC. Histopathological correlates with survival in reoperated glioblastomas. J Neurooncol. 2013; 113(3):485–93.
7. Young RJ, Gupta A, Shah AD, Graber JJ, Chan TA, Zhang Z, et al. MRI perfusion in determining pseudoprogression in patients with glioblastoma. Clin Imaging. 2013; 37(1):41–9.
8. Stuplich M, Hadizadeh DR, Kuchelmeister K, Scorzin J, Filss C, Langen KJ, et al. Late and prolonged pseudoprogression in glioblastoma after treatment with lomustine and temozolomide. J Clin Oncol. 2012; 30(21):180–3.
9. Linhares P, Carvalho B, Figueiredo R, Reis RM, Vaz R. Early Pseudoprogression following Chemoradiotherapy in Glioblastoma Patients: The Value of RANO Evaluation. J Oncol. 2013; 5:690585
10. Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol. 2008; 26(13):2192–7.
11. Abbasi AW, Westerlaan HE, Holtman GA, Aden KM, van Laar PJ, van der Hoorn A. Incidence of Tumour Progression and Pseudoprogression in High-Grade Gliomas: a Systematic Review and Meta-Analysis. Clin Neuroradiol. 28(3):401–11.
12. Chaskis C, Neyns B, Michotte A, De Ridder M, Everaert H. Pseudoprogression after radiotherapy with concurrent temozolomide for high-grade glioma: clinical observations and working recommendations. Surg Neurol. 2009; 72(4):423–8.
13. Chu HH, Choi SH, Ryoo I, Kim SC, Yeom JA, Shin H, et al. Differentiation of True Progression from Pseudoprogression in Glioblastoma Treated with Radiation Therapy and Concomitant Temozolomide: Comparison Study of Standard and High- *b* -Value Diffusion-weighted Imaging. Radiology. 2013; 269(3):831–40.
14. Zhao X, Chen R, Liu M, Feng J, Chen J, Hu K. Remodeling the blood–brain barrier microenvironment by natural products for brain tumor therapy. Acta Pharm Sin B. 2017; 7(5):541–53.
15. Vajkoczy P, Menger MD. Vascular microenvironment in gliomas. J Neurooncol. 2000; 50(1–2):99–108.
16. Hellinger É, Veszelka S, Tóth AE, Walter F, Kittel Á, Bakk ML, et al. Comparison of brain capillary endothelial cell-based and epithelial (MDCK-MDR1, Caco-2, and VB-Caco-2) cell-based surrogate blood-brain barrier penetration models. Eur J Pharm Biopharm. 2012; 82(2):340–51.
17. Veszelka S, Tóth AE, Walter FR, Datki Z, Mózes E, Fülöp L, et al. Docosahexaenoic acid reduces amyloid-β induced toxicity in cells of the neurovascular unit. J Alzheimer's Dis. 2013; 36(3):487–501.
18. Jähne EA, Eigenmann DE, Culot M, Cecchelli R, Walter FR, Deli MA, et al. Development and validation of a LC-MS/MS method for assessment of an anti-inflammatory indolinone derivative by in vitro blood-brain barrier models. J Pharm Biomed Anal. 2014; 98:235–46.
19. Tóth AE, Walter FR, Bocsik A, Sántha P, Veszelka S, Nagy L, et al. Edaravone protects against methylglyoxal-induced barrier damage in human brain endothelial cells. PLoS One. 2014; 9(7):1–14.

Утицај ендотелијалне хиперплазије на појаву псеудопрогресије код болесника са глиобластомом

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САЖЕТАК

Увод/Циљ Псеудопрогресија представља појаву лезије која се пребојава контрастом после хемоирадијације и која не прогредира или се повлачи без промене терапије. Сам феномен има изразит клинички значај зато што у великој мери утиче на даље терапијске поступке, али критеријуми за њено дијагностиковање још увек нису јасно дефинисани. Циљ овог истраживања је испитивање утицаја ендотелијалне хиперплазије на настанак псеудопрогресије.

Методе Испитивано је 106 болесника који су оперисани због глиобластома у Клиничком центру Србије у периоду од 2010. до 2012. године, и који су после тога лечени Ступовим протоколом. Снимање пре и после лечења је утврђено критеријумом *RANO*. Промене које су биле стабилне или су се повукле дефинисане су као псеудопрогресија, док су лезије које су прогредирале окарактерисане као праве прогресије. Ендотелијална хиперплазија је утврђена после патохистолошког прегледа хематоксилином и еозином.

Резултати Псеудопрогресија је регистрована код 32 (30,2%) болесника. Код 51 (48,1%) узорка ткива глиобластома уочена је ендотелијална хиперплазија. Од свих болесника код којих је регистрована псеудопрогресија, њих 28 (87,5%) имало је ендотелијалну хиперплазију. Код болесника који нису имали псеудопрогресију, њих 51 (68,9%) није имало ни ендотелијалну хиперплазију. Код болесника који су испољили псеудопрогресију било је значајно више болесника који су у патохистолошком налазу имали описану и ендотелијалну хиперплазију, док код болесника без псеудопрогресије има више случајева без ендотелијалне хиперплазије ($\chi^2 = 26{,}269$, $p < 0{,}01$).

Закључак С обзиром на то да за сада не постоји прецизна дијагностичка метода која би са сигурношћу утврдила да ли се ради о псеудопрогресији, присуство ендотелијалне хиперплазије би могло да укаже на већу вероватноћу појаве псеудопрогресије, што би се могло користи у свакодневном клиничком раду. Ипак, ради доношења дефинитивних закључака, сматрамо да је неопходно спровести проспективне контролисане студије на већем узорку.

Кључне речи: глиобластом; ендотелијална хиперплазија; псеудопрогресија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The role of regional anesthesia in the postoperative analgesia in pediatric patients

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SUMMARY
Introduction/Objective Pain is a disturbing experience associated with existing or potential tissue damage, with a sensory, emotional, cognitive, and social component.
The aim of this study was to show the efficiency of regional anesthetic techniques in postoperative pain in children.
Methods The retrospective cohort study was conducted on a group of 564 pediatric patients during the period from 2013 to 2016. Types of regional anesthesia were classified into the following six groups: caudal, epidural, spinal block, upper limb blocks, lower limb blocks, and truncal nerve block. From statistical methods, we used descriptive statistical methods of absolute and relative numbers, measurements of variability, central tendencies for numerical features, and methods of inferential statistics. We used the χ^2 test for the attributive features of observations.
Results In relation to the postoperative time when an analgesic was required, a statistically significant difference was observed in the age of children ($p = 0.000$), disease diagnosis ($p = 0.000$), type of block ($p = 0.000$), type of local anesthetic ($p = 0.000$), and type of anesthesia or sedation preoperatively ($p = 0.005$).
Conclusion Postoperative analgesia was most needed by older children and children who were awake during surgery. Children with injuries and tumors need postoperative analgesia the earliest. The longest postoperative analgesia was recorded in patients who received caudal block. The longest postoperative analgesia can be seen in patients who received levobupivacaine, bupivacaine or levobupivacaine combined with lidocaine to perform the block.
Keywords: regional anesthesia; child; pediatric patient; postoperative analgesia

INTRODUCTION

Pain, according to the new definition, is a disturbing experience associated with existing or potential tissue damage, with a sensory, emotional, cognitive and social component [1]. During childhood, pain has numerous adverse effects – it can prolong hospital stay and even increase incidence of death due to the onset of the systemic inflammatory response [2]. Inadequate treatment of acute pain is one of the important prerequisites for the development of chronic pain. Repetition of painful procedures determines the threshold for pain for the whole life [3].

In pediatric patients, less local anesthetic concentration is more effective than in adults [4]. The effect of the block is faster and shorter. In children under one year of age, nerve fibers are thinner, myelination is scarce, and Ranvier's knots are closer. The volume of distribution is higher, clearance is smaller, and the free drug fraction is higher, so the doses are almost the same as in adults [5, 6, 7]. Cytochrome CYP1A2 that catalyzes the metabolism of ropivacaine matures around the age of 4–7 years, and CYP3A4/7 that catalyzes the metabolism of levobupivacaine matures at the end of the first year [4].

The goal of analgesia in the postoperative period is to reduce or eliminate pain with minimal additional harmful effects. Adequate postoperative analgesia, especially during the first 48 hours, reduces the organism stress response to the surgical procedure, thereby affecting endocrine, metabolic, and inflammatory changes, which improves the outcome of surgical treatment [3, 8–11].

METHODS

We performed retrospective review of pediatric patients (1–14 years), who had been operated on in regional anesthesia techniques, between January 2013 and December 2016. The patients were divided into the following four groups: children younger than three years, those aged 3–7 years, those 7–14 years old, and children

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older than 14 years. All the patients were classified by the American Association of Anesthesiologists classification and all belonged to groups 1–3.

Regional anesthesia techniques were performed during general anesthesia, analgosedation or in the waking state of the patients. We used bupivacaine, lidocaine, and levobupivacaine, as well as combinations that consisted of lidocaine with levobupivacaine and lidocaine with bupivacaine.

Types of applied regional anesthesia techniques were classified into the following six groups: caudal block, epidural, spinal, upper limb blocks, lower limb blocks, and truncal nerve block. The diagnosis of patients involved in the study included injuries, tumors, congenital anomalies, arteriovenous fistula, and appendicitis. Pulse, blood pressure, and oxygen saturation were used as standard anesthesia monitoring.

Postoperatively, the patients were diagnosed with pain based on a scale for age-related pain by nurses who were trained for this follow-up after two, four, six, eight, 10, 12, and 14 hours. The time when analgesia caused by regional anesthesia became insufficient and when it was necessary to apply the analgesic was recorded, and the patients were then classified accordingly into one of the following groups: < 3 hours, 3–6 hours, 6–9 hours, 9–12 hours, > 12 hours.

Also, we analyzed the postoperative occurrence and we measured the intensity of pain by different methods in different ages of children: in children ≤ 12 months old we used the CRIES scale, in children ≤ 3 years old by using FLACC scale, in children 3–5 years old we used Wong scale with facial expressions, and in older children we used linear scales [3, 12, 13].

The exclusion criteria were the following: patients with hypotension, thrombocytopenia, coagulopathy, sepsis, myelodysplasia, and sacral dysgenesis.

The study was approved by the Belgrade University Children's Hospital Ethics Committee (No. 26/343-017).

Descriptive statistical methods of absolute and relative numbers were used for attribute observation marks, variability measures, central tendencies for numerical labels and methods of inferential statistics. The selection of tests for numerical markings depended on the distribution of data. We used the χ^2 test for the attributive features of observations. Statistical analysis was performed with SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study was conducted over a period of four years, between January 2013 and December 2016. The summarized patient characteristics, sex, age, indications for surgery, type of local anesthetics, and regional anesthesia techniques are shown in Table 1.

There was no statistically significant difference between type of regional anesthesia techniques and pulse, blood pressure, and oxygen saturation during the operation. After administration of the block as well as during the surgical procedure, all vital signs were within the limits of the reference values for the patient's age in all our patients.

Table 1. Patient characteristics, indications for surgery, type of local anesthetics, and regional anesthesia techniques

Number (%)	Male	377 (66.8%)
	Female	187 (33.2%)
Age (years)	< 3	84 (14.9%)
	3–7	139 (24.6%)
	7–14	224 (39.7%)
	> 14	117 (20.7%)
Diagnosis	Tumors	101 (17.9%)
	Injury	201 (35.6%)
	Arteriovenous fistula	3 (0.5%)
	Appendicitis	21 (3.7%)
	Congenital anomaly	237 (42%)
Type of regional anesthesia techniques	Caudal block	168 (29.8%)
	Epidural	20 (3.5%)
	Spinal	12 (2.1%)
	The upper limb block	179 (31.7%)
	The lower limb block	163 (28.9%)
	The hull block	22 (3.9%)
Type of local anesthetic	Bupivacaine	213 (37.8%)
	Lidocaine	13 (2.3%)
	Bupivacaine + Lidocaine	287 (50.9%)
	Levobupivacaine	28 (5%)
	Levobupivacaine + Lidocaine	23 (4.1%)

There was a significant difference between the age of children and the reduction in the time of postoperative analgesia (Table 2). There was no statistically significant difference between the sex and the time of administration of analgesics postoperatively.

There was a significant difference between the time for postoperative analgetic requirements and the preoperative diagnosis. The shortest time to administer analgetics was in patients with injuries and tumors. Arteriovenous fistula was made in only three patients and the first dose of analgesics was given to all 3–6 hours after intervention (Table 3).

There was a significant difference between the type of block and the first dose of analgetics. The longest postoperative analgesia was recorded in the group with the caudal

Table 2. Time to first dose of postoperative analgesic in relation to age

Age (years)	< 3 hours	3–6 hours	6–9 hours	9–12 hours	> 12 hours	Significance
< 3	6 (7.1%)	9 (10.7%)	28 (33.3%)	33 (39.3%)	8 (9.5%)	[a]p = 0.000*
3–7	9 (6.05%)	27 (19.4%)	50 (36%)	46 (33.1%)	7 (5%)	
7–14	20 (8.9%)	72 (32.1%)	73 (32.6%)	44 (19.6%)	15 (6.7%)	
> 14	14 (12%)	42 (35.9%)	30 (25.6%)	23 (19.7%)	8 (6.8%)	

*Statistically significant difference;
[a]χ^2 test

Table 3. Time to first dose of postoperative analgesic in relation to diagnosis

Diagnosis	< 3 hours	3–6 hours	6–9 hours	9–12 hours	> 12 hours	Significance
Tumors	14 (13.9%)	29 (28.7%)	27 (26.7%)	21 (20.8%)	10 (9.9%)	[a]p = 0.000*
Injury	19 (9.5%)	79 (39.3%)	61 (30.3%)	35 (17.4%)	7 (3.5%)	
AV fistula	0 (0%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	
Appendix	0 (0%)	0 (0%)	5 (23.8%)	14 (66.7%)	2 (9.5%)	
Congenital anomalies	16 (6.8%)	39 (16.5%)	88 (37.1%)	75 (31.6%)	19 (8%)	

AV – arteriovenous fistula;
*statistically significant difference;
[a]χ^2-test

Table 4. Time to first dose of postoperative analgesic in relation to type of regional anesthesia

Block	< 3 hours	3–6 hours	6–9 hours	9–12 hours	> 12 hours	Significance
Caudal	8 (4.8%)	19 (11.3%)	71 (42.3%)	59 (35.1%)	11 (6.5%)	[a]p = 0.000*
Epidural	6 (30%)	6 (30%)	5 (25%)	3 (15%)	0 (0%)	
Spinal	1 (8.3%)	3 (25%)	5 (41.7%)	3 (25%)	0 (0%)	
Upper limb	20 (11.2%)	75 (41.9%)	51 (28.5%)	28 (15.6%)	5 2.8%)	
Lower limb	14 (8.6%)	47 (28.8%)	44 (27%)	38 (23.3%)	20 (12.3%)	
Truncal	0 (0%)	0 (0%)	5 (22.7%)	15 (68.2%)	2 (9.1%)	

*Statistically significant difference;
[a]χ^2 test

and truncal nerve blockade. Spinal anesthesia and blocks of the lower extremities were the following successive blocks in terms of postoperative analgesia. The shortest analgesic time was observed in patients with epidural and upper limb blocks (Table 4).

There was a significant difference between the type of local anesthetic and the first dose of analgesics that was given postoperatively. Longer postoperative analgesia was seen in children in whom the levobupivacaine, bupivacaine or levobupivacaine combined with lidocaine was used to perform the block.

There were significant differences between the blocks under sedation and blocks under general anesthesia and the first time of giving postoperative analgesia. The shortest time of giving the first analgesic postoperatively was in patients who were awake during surgery.

DISCUSSION

Only a few decades ago it was considered that pain was the normal "price" to be paid for successfully performed surgery. It was also thought that the newborn did not feel pain, and surgical interventions during the first few days of life were mostly performed without any anesthesia [14]. Today, we consider that surgical pain not only hurts but can have effects that compromise recovery. Since it is generally accepted that even small children feel pain, analgesia must be ensured for all and after all painful procedures. It has been proven that adequate perioperative analgesia reduces the metabolic response to the trauma, the possibility of chronic pain, and morbidity and mortality [15]. It has been clearly demonstrated that poor postoperative pain control in children leads to emotional disorders [16]. It can also lead to more immediate complications – hypoventilation after thoracotomy, an increase in arterial and intracranial pressure.

Regional anesthesia can provide good perioperative pain control. The pre-surgery block contributes to intraoperative analgesia and reduces the need for other analgetics. There has long been evidence that postoperative pain is lower if general anesthesia is given in combination with the regional one, although regional anesthesia has not yet found full application in pediatric patients [17].

The concept that regional and general anesthesia are complementary rather than competitive is fundamental to comprehensively comprehending the role of regional anesthesia in children and dates back to the early 1990s [18].

In our study, regional anesthesia was performed in pediatric patients, in analgosedation or in general anesthesia. We herein show that postoperative analgesia was needed earlier in patients who were conscious.

All of our patients were completely hemodynamic and respiratory stable during and after the operation, which contributed to a faster release from the hospital. It has been explained long time ago why even very small children maintain hemodynamic stability even after central blocks – it has been explained by the immaturity of the sympathetic nervous system, relatively lower volume of lower extremities, and compensatory vasoconstriction in unblocked blood vessels [19, 20].

In our study, we show that the earliest use of postoperative analgetics was needed in children who were operated on due to injuries or tumors. This demonstrates how much the psychological component of pain in children, i.e. suffering due to the realization of the severity of the disease or potential disfiguration, is important in the feeling of pain.

In this study, the effect of the dose and volume of local anesthetics on postoperative analgesia were not studied, because doses and volume were applied on the basis of consensus adopted [9]. However, statistically significant longer analgesia was achieved when long-acting local anesthetics (levobupivacaine, bupivacaine or levobupivacaine in combination with lidocaine) were used. It is understand-

able that the duration of the block will depend on the pharmacodynamics of the drug used.

Analgesia was the longest after the caudal and truncal nerve blockade, which is in accordance with the literature data [4]. Shorter analgesia was after spinal anesthesia and blocks of the lower extremities. One of the described defects in spinal anesthesia during childhood is just shorter time [4]. It is not entirely clear why the shortest analgesia was after epidural anesthesia. The only explanation is that this type of block was applied in the eldest age group, where additional analgesia was needed at the earliest, most likely because of the psychological, social, and cognitive component of the pain affected [21].

However, if we analyze the literature data on the isolated influence of age on pain, they are extremely contradictory – from the statement that the feeling of pain is the greatest in the youngest population, to the assertion that age is not significant [21, 22].

There are many controversies in the literature concerning the correlation between pain and the sex [21, 23, 24]. In our study, we did not find statistically significant differences between the sex and pain. One of the reasons may be that we had significantly more boys than girls, or that the influence of the sex on pain occurs much later.

Good postoperative analgesia reduces the risk for postoperative anxiety and delirium, which fall into the group of significant complications of anesthesia, especially in the pediatric population [25]. The costs were significantly reduced due to the reduced need for intraoperative use of expensive inhalation anesthetics, and postoperatively due to the reduced need for the addition of analgesics. This decreased the potential side effects of systemic analgesics that could also prolong hospitalization.

CONCLUSION

Good analgesia reduces the need for postoperative analgesics and secures better comfort in the postoperative period. Postoperative analgesia is needed the most by older children and by patients who had been awake during surgery, probably due to the most developed cognitive, psychological and social component of the pain. Children with injuries and tumors need postoperative analgesia the earliest. The longest postoperative analgesia was recorded in patients who received caudal block. The longest postoperative analgesia can be seen in patients who received levobupivacaine, bupivacaine or levobupivacaine combined with lidocaine to perform the block.

The goal of a physician should always be to minimize the psychological and physical trauma to the patient, regardless of how young and immature he is. From the ethical point of view, it is not justifiable to allow a child to suffer pain, when simple and safe techniques of regional anesthesia can easily complement or replace conventional general anesthesia.

Conflict of interest: None declared.

REFERENCES

1. Williams AC, Craig KD. Updating the definition of pain. Pain. 2016; 157(11):2420–3.
2. Ahmedi A, Bazargan-Hejazi S, Zadie ZH, Euasobhon P, Ketumam P, Karbasfrushan A, et al. Pain management in trauma: A review study. J Inj Violence Res. 2016; 8(2):89–98.
3. Kehlet H, Holte K. Effect of postoperative analgesia on surgical outcome. Br J Anaesth. 2001; 87(1):62–72.
4. Jöhr M. Regional anaesthesia in neonates, infants and children: an educational review. Eur J Anaest. 2015; 32(5):289–97.
5. Rosenberg PH, Veering BT, Urmey WF. Maximum recommended doses of local anesthetics: a multifactorial concept. Reg Anesth Pain Med. 2004; 29(6):564–75.
6. Palmon SC, Lloyd AT, Kirsch JR. The effect of needle gauge and lidocaine pH on pain during intradermal injection. Anesth Analg. 1998; 86(2):379– 81.
7. Scarfone RJ, Jasani M, Gracely EJ. Pain of local anesthetics: rate of administration and buffering. Ann Emerg Med. 1998; 31(1):36–40.
8. Moraca RJ, Sheldon DG, Thirlby RC. The role of epidural anesthesia and analgesia in surgical practice. Ann Surg. 2003; 238(5):663–73.
9. Waurick R, Van Aken H. Update in thoracic epidural anaesthesia. Best Pract Res Clin Anaesthesiol. 2005; 19(2):201–13.
10. Pasero C. Improving postoperative outcomes with epidural analgesia. J Perianesth Nurs. 2005; 20(1):51–5.
11. Liu SS, Wu CL. Effect of postoperative analgesia on major postoperative complications: a systematic update of the evidence. Anesth Analg. 2007; 104(3):689–702.
12. The Faces Pain Scale – Revised. Pediatric Pain Sourcebook of Protocols, Policies and Pamphlets. 7 August 2007.
13. Hartrick CT, Kovan JP, Shapiro S. The numeric rating scale for clinical pain measurement: a ratio measure? Pain Pract. 2003; 3(4):310–6.
14. Williamson PS, Williamson ML. Physiologic stress reduction by local anesthetic during newborn circumcision. Pediatrics. 1983; 71(1):36-40.
15. Zeltzer LK, Anderson CT, Schechter NL. Pediatric pain: current status and new directions. Curr Probl Pediatr. 1990; 20(8):409–86.
16. Vernon DT, Schulman JL, Foley JM. Changes in children's behavior after hospitalization. Am J Dis Child. 1966; 111(6):581–93.
17. Tverskoy M, Cozacov C, Ayache M, Bradley EL Jr, Kissin I. Postoperative pain after inguinal herniorrhaphy with different types of anesthesia. Anaesth Analg. 1990; 70(1):29–35.
18. Flecher B, Owen J. Conclusion. In: Pediatric Regional Anesthesia. Dalens BJ. CRC Press, Inc. Boca Ration, Florida. 1990:477.
19. Gootman PM, Gootman N, Buckley BJ. Maturation of central autonomic control of the circulation. Fed Proc. 1983; 42(6):1648–55.
20. Payen D, Ecoffey C, Carli P, Dubousset AM. Pulsed doppler ascending aortic, carotid, brachial and femoral artery blood flows during caudal anesthesia in infants. Anesthesiology. 1987; 67(5):681–5.
21. Wander LD, Scipio CD, Hirsh AT, Torres CA, Robinson ME. The perception of pain in others: how gender, race and age influence pain expectations. J Pain. 2012; 13(3):220–7.
22. Ranger M, Johnston CC, Anand KJ. Current controversies regarding pain assessment in neonates. Semin Perinatol 2007; 31(5):283–8.
23. Pieretti S, Di Giannuario A, Di Giovannandrea R, Marzoli F, Piccaro G, Minosi P, et al. Gender differences in pain and its relief. Ann Ist Super Sanita. 2016; 52(2):184–9.
24. Vernon DT, Schulman JL, Foley JM. Changes in children's behavior after hospitalization. Am J Dis Child. 1966; 111(6):581–93.
25. Suresh S, Schaldenbrand K, Wallis B, De Oliveira GS Jr. Regional anaesthesia to improve pain outcomes in paediatric surgical patients: a qualitative systematic review of randomized controlled trials. Br J Anaesth. 2014; 113(3):375–90.

Улога регионалне анестезије у постоперативној аналгезији педијатријских болесника

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САЖЕТАК

Увод/Циљ Бол представља узнемирујуће искуство које је повезано са постојећим или могућим оштећењем ткива, са сензорном, емоционалном, когнитивном и социјалном компонентом.

Циљ ове студије је приказ ефикасности техника регионалне анестезије на постоперативни бол код деце.

Методе Ретроспективна кохортна студија је спроведена на групи од 564 педијатријских болесника у периоду од 2013. до 2016. године. Врсте регионалне анестезије су класификоване у шест група: каудална, епидурална, спинална, блокови горњих екстремитета, доњих екстремитета и блок трупа. Од статистичких метода користили смо дескриптивне статистичке методе апсолутних и релативних бројева за атрибутивна обележја посматрања, мере варијабилитета, централне тенденције за нумеричка обележја и методе инференцијалне статистике. Избор тестова за нумеричка обележја посматрања зависиће од расподеле података. За атрибутивна обележја посматрања користили смо χ^2 тест.

Резултати У односу на постоперативно време када је био потребан аналгетик, статистички значајна разлика уочена је у узрасту деце ($p = 0,000$), дијагнози болести ($p = 0,000$), врсти блока ($p = 0,000$), врсти коришћеног локалног анестетика ($p = 0,000$), као и врсти периоперативне анестезије или седације ($p = 0,005$).

Закључак Постоперативна аналгезија је најпотребнија старијој деци и деци која су била будна током хируршке интервенције. Најранија постоперативна аналгезија је потребна деци са повредама и туморима. Најдужа постоперативна аналгезија је забележена код болесника који су добили каудални блок, као и болесника који су примили левобупивакаин, бупивакаин или левобупивакаин у комбинацији са бупивакаином за извођење блока.

Кључне речи: регионална анестезија; дете; педијатријски болесник; постоперативна аналгезија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Long-term attitude towards follow-up colposcopy in women of reproductive age after excisional treatment for cervical dysplasia

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SUMMARY

Introduction/Objective Very little is known about the factors influencing women's attitude towards colposcopy follow-up after cervical treatment.
The aim of the study was to investigate the long-term attitude to follow-up colposcopy in women of reproductive age after cervical excision and to evaluate if their attitude was related to their anxiety and depression levels.

Methods Women treated with cervical excision were interviewed after a follow-up colposcopy visit. Their socio-demographic and clinical characteristics were recorded. All women filled in the Beck's anxiety and depression inventory.

Results A total of 160 women were divided into the study group of 42 (26.3%) women who felt discomfort during follow-up colposcopy and the control group of 118 (73.7%) women who did not report such feelings. The mean age of the total sample was 35.3 ± 5.4 years with median time after treatment being five years (range: 2–18). Women in the study group had a significantly lower BMI values, had higher rates of nulliparity and nulligravidity, were more often single or living alone, and had significantly changed their attitude towards condom use after treatment. Beck's anxiety and depression scores were significantly higher in the study group. Multivariate analysis showed that independent predictors of discomfort during follow-up colposcopy were anxiety levels (OR: 1.06; 95% CI: 1–1.12), living alone or without a partner (OR: 2.65; 95% CI: 1.08–6.55), and the change in their practice of condom use after treatment (OR: 2.69; 95% CI: 1.02–7.07).

Conclusion Almost one third of women after excisional treatment reported discomfort during their follow-up colposcopy. These women exhibited higher levels of anxiety.

Keywords: cervical dysplasia; conization; follow-up; colposcopy; anxiety; depression

INTRODUCTION

The human papillomavirus (HPV) infection affects up to 80% of females during their lifetime [1, 2]. Although most infections are transient, 10–20% of women develop a persistent HPV infection which could cause squamous intraepithelial lesions (SIL) [1]. Low-grade squamous intraepithelial lesions (LSIL) have a high potential for spontaneous regression, while high-grade squamous intraepithelial lesions (HSIL) are to be considered precursor lesions for cervical carcinoma (CC) [3].

Most often, SIL is diagnosed during the reproductive age, when the majority of women have not yet completed their family [4, 5, 6]. Women with HSIL are treated with cervical excision to prevent disease progression into CC, while those with LSIL are treated only in cases of persistent lesions. Following treatment, women are at an increased risk for recurrent SIL and/or CC as compared to the general population thus requiring long-term surveillance [7, 8]. Little is known about the factors influencing the long-term attitude towards post-treatment follow-up in women who have had excisional cervical treatment for SIL [9, 10].

The objective of our study was to investigate the long-term attitude towards colposcopy follow-up in women of reproductive age at two or more years after excisional cervical treatment of SIL through the process of an interview after a routine follow-up visit at the colposcopy clinic and to evaluate if their attitude was related to their anxiety and depression levels.

METHODS

This cross-sectional study took place in the Colposcopy Unit of the Clinic for Gynecology and Obstetrics at the Clinical Center of Serbia between April 2014 and October 2016. Our colposcopy service provides long-term follow-up to patients treated for SIL in our institution. The cohort of women in the presented study is the same cohort that was used for the cross-cultural adaptation and psychometric validation

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of the FACIT-CD questionnaire in Serbian women that was published earlier [11].

We included women who were between 18 and 45 years old, and who received cervical treatment in the form of either cold-knife-conization (CKC) or large loop excision of the transformation zone (LLETZ). The cervical treatment was performed more than two years prior to this study and squamous intraepithelial neoplasia lesions was histologically confirmed in all participants. We excluded women with a postmenopausal status, who were pregnant, with a history of previous cervical treatment, who had prior operation on the reproductive organs except for caesarean section delivery, and with acute gynecological or other major diseases that could possibly affect their psychological well-being. Women with glandular cervical abnormalities and invasive cervical disease were also excluded.

Participants were interviewed by the first author after their routine follow-up colposcopy examination. The following socio-demographic and clinical data were collected: age, period from treatment, level of education, employment, marital status, and condom use after the treatment, age of menarche, reproductive history, and age at the time of treatment. In addition, the body mass index (BMI) of all participants was calculated. The following clinical data were also obtained: indication for the excision (colposcopy/cytology/punch biopsy histopathology), type of treatment (LLETZ/CKC), final histopathology result, postoperative complications, cytology follow-up results, and postoperative infertility.

All participants filled in the Serbian version of Beck's anxiety (BAI) and depression (BDI) inventory. Both inventories consisted of 21 questions with a score assigned to each item (range: 0–3). Minimal levels of anxiety correspond with a BAI score of ≤ 7, while minimal levels of depression correspond with a BDI score of ≤ 9, with increasing scores indicating higher levels of anxiety or depression [12].

All women prior to their interview gave written informed consent for the study. The study was approved by the Ethics Committee of the Clinical Centre of Serbia on March 20, 2014 (decision No 541/4).

Statistical data analysis was performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Results were presented as frequency (percent), median (range) and mean ± SD (standard deviation). For parametric data independent samples t-test was used to test differences between groups. For numeric data with non-normal distribution and ordinal data, Mann-Whitney U test was used. χ^2 test or Fisher's exact test was used to test differences between nominal data (frequencies). Predictors of a woman's attitude towards colposcopy follow-up were analyzed by univariate and multivariate logistic regression. All p values under 0.05 were considered significant.

RESULTS

A total of 160 patients were divided into two groups: the study group of 42 (26.3%) women feeling discomfort during the follow-up colposcopy examination and the control group of 118 (73.7%) women who did not.

The mean age of the participants was 35.3 ± 5.4 years (range: 22–44), with a median equal to 36 at interview. The mean time after the excision was 4.9 ± 3 years (range: 2–18) with a median of five years. Women in the study group had a significantly lower BMI, were more frequently nulliparous and nulligravida, single or living alone, and

Table 1. Clinical and socio-demographic data of the patients at the time point of their interview

Data		Total n = 160	Study group n = 42	Control group n = 118	p
Clinical data					
Age, years (mean ± SD)		35.3 ± 5.4	34.1 ± 5.7	35.8 ± 5.2	0.080
Years after treatment (mean ± SD)		4.9 ± 3	4.7 ± 3.2	5 ± 3	0.492
BMI, kg/m^2 (mean ± SD)		22.2 ± 3	21.4 ± 2.2	22.5 ± 3.2	**0.039**
Menarche, years (mean ± SD)		13.2 ± 1.7	13.2 ± 2	13.1 ± 1.6	0.796
Abortions, n (%)	0	104 (65)	29 (69)	75 (63.6)	0.522
	≥ 1	56 (35)	13 (31)	43 (36.4)	
Deliveries, n (%)	0	59 (36.9)	22 (52.4)	37 (31.4)	**0.015**
	≥ 1	101 (63.1)	20 (47.6)	81 (68.8)	
Gravidity, n (%)	0	44 (27.5)	18 (42.9)	26 (22)	**0.009**
	≥ 1	116 (72.5)	24 (57.1)	92 (78)	
Socio-demographic data					
Education, n (%)	≤ 12 years	84 (52.5)	23 (54.8)	61 (51.7)	0.732
	> 12 years	76 (47.5)	19 (45.2)	57 (48.3)	
Employment, n (%)	yes	33 (20.6)	8 (19)	25 (21.2)	0.769
	no	127 (79.4)	34 (81)	93 (78.8)	
Actual marital status, n (%)	single or living alone	54 (33.7)	22 (52.4)	32 (27.1)	**0.003**
	married or coupled	106 (66.3)	20 (46.7)	86 (72.9)	
Condom use after the treatment, n (%)	same	135 (84.4)	31 (73.8)	104 (88.1)	**0.028**
	changed	25 (15.6)	11 (26.2)	14 (11.9)	

BMI – body mass index; SD – standard deviation

Table 2. Perioperative and postoperative characteristics of the patients

Characteristics		Total n = 160	Study group n = 42	Control group n = 118	p
Age at surgery, years (mean ± SD)		30.4 ± 5	29.4 ± 5.7	30.8 ± 4.8	0.112
Indication for surgery, n (%)	colposcopy	10 (6.3)	2 (4.8)	8 (6.8)	0.857
	cytology	57 (35.6)	16(38.1)	41 (34.7)	
	histology	93 (58.1)	24 (57.1)	69 (58.5)	
Type of treatment, n (%)	LLETZ	113 (70.6)	35 (83.3)	78 (66.1)	**0.035**
	CKC	47 (29.4)	7 (16.7)	40 (33.9)	
Final histopathology, n (%)	LSIL	43 (26.9)	13 (31)	30 (25.4)	0.488
	HSIL	117 (73.1)	29 (69)	88 (76.4)	
Postoperative complications, n (%)	no	134 (83.8)	32 (76.2)	102 (86.4)	0.122
	yes	26 (16.3)	10 (23.8)	16 (13.6)	
Abnormal cytology during follow-up, n (%)	no	126 (78.8)	32 (76.2)	94 (79.7)	0.637
	yes	34 (21.3)	10 (23.8)	24 (20.3)	
Postoperative infertility, n (%)	no	133 (83.1)	37 (88.1)	96 (81.4)	0.317
	yes	27 (16.9)	5 (11.9)	22 (18.6)	

LLETZ – large loop excision of the transformation zone; CKC – cold knife conization; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesion

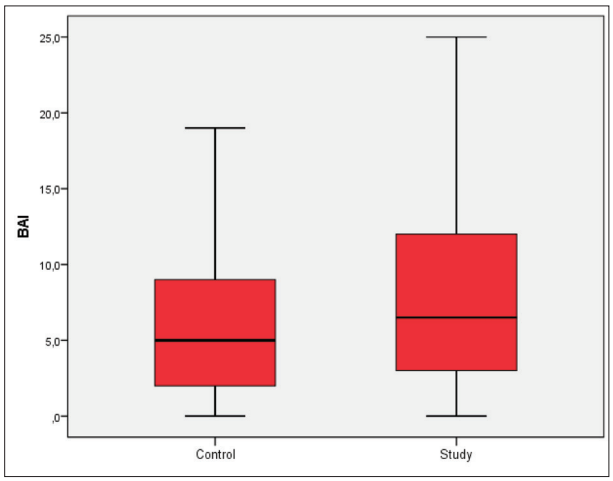

Figure 1. Beck anxiety inventory scores in the study and control group

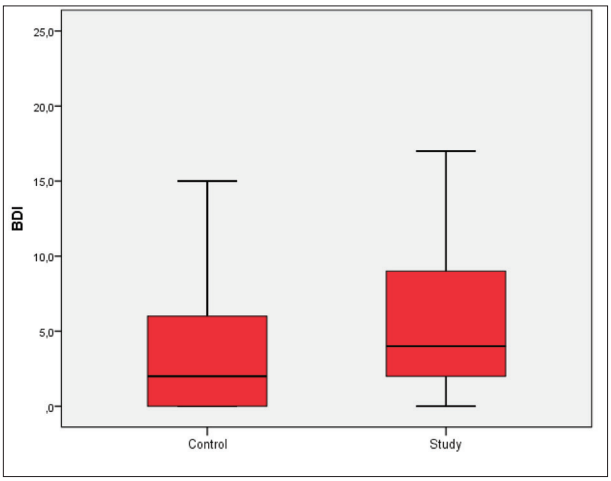

Figure 2. Beck depression inventory scores in the study and control group

had changed their attitude towards condom use after the treatment. Four (1.9%) women had BMI ≥ 30. There were no significant differences detected between the two groups when comparing other characteristics. Table 1 shows the clinical and socio-demographic data of the women at the time point of their interview.

The perioperative and postoperative characteristics of the patients are presented in Table 2. In terms of the type of treatment, there was a significant difference among the groups, as women in the study group mainly had LLETZ. CKC was more frequently performed in parous woman, as only six (12.8%) woman who underwent CKC were nulliparous in comparison to 53 (46.9%) in the LLETZ group ($p < 0.001$; data not shown). In terms of other characteristics, there were no statistically significant differences between the groups.

The majority of the participants had BAI scores ≤ 7 (n = 99 women; 61.9%) and the majority of the participants had BDI scores ≤ 9 (n = 137 women; 85.6%). The average BAI score was 6.92 ± 6.20 in the total sample, 8.81 ± 7.16 in the study group and 6.25 ± 5.70 in the control group, respectively (Figure 1). The average BDI score was 4.37 ± 4.89 in the total sample, 5.74 ± 4.92 in the study group and 3.88 ± 4.81 in the control group, respectively (Figure 2). Significant differences in Beck's anxiety (BAI) and depression (BDI) inventory median scores were detected when comparing the study and the control group: the median BAI score was 6.5 (IQR 9.3, range: 0–25) in the study group and 5 (IQR 7, range: 0–25) in the control group ($p = 0.041$). The median BDI score was 4 (IQR 7.3, range: 0–17) in the study group and 2 (IQR 6, range: 0–25) in the control group ($p = 0.012$). We further compared BAI and BDI scores regarding SIL grade, to evaluate if the anxiety and depression levels that were possibly associated with the SIL grade biased our results. There were no differences between the patients with LSIL and patients with HSIL groups in relation to median BAI and BDI scores (Table 3).

We further compared BAI and BDI scores regarding SIL grade, to evaluate if the anxiety and depression possibly associated with the SIL grade biased our results.

Multivariate logistic regression analysis showed that discomfort during follow-up colposcopy was independently

Table 3. BAI and BDI questionnaire scores in relation to the SIL grade

Score		Total n = 160	LSIL n = 43	HSIL n = 117	p
BAI	≤ 7	99 (61.9%)	24 (58.8%)	75 (64.9%)	0.339
	≥ 8	61 (38.1%)	19 (44.2%)	42 (38.1%)	
BDI	≤ 9	137 (85.6%)	40 (93%)	97 (85.6%)	0.105
	≥ 10	23 (14.4%)	3 (7%)	20 (14.4%)	

BAI – Beck Anxiety Inventory; BDI – Beck Depression Inventory; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesion

Table 4. Predictors of the discomfort during colposcopy

Predictors	B	p	OR	95% CI	
				Lower limit	Upper limit
Number of deliveries	0.169	0.552	1.18	0.68	2.07
BMI (kg/m^2)	-0.129	0.089	0.88	0.76	1.02
BAI score	0.058	**0.049**	1.06	1.00	1.12
Barrier contraception use after treatment (changed/same)	0.988	**0.045**	2.69	1.02	7.07
Marital status (single or other/married or coupled)	0.976	**0.034**	2.65	1.08	6.55
Type of treatment (CKC/LLETZ)	-0.864	0.100	0.42	0.15	1.18

BMI – body mass index; BAI – Beck Anxiety Inventory; B – regression coefficient; OR – odds ratio; LLETZ – large loop excision of the transformation zone; CKC – cold knife conization

associated with the change in condom use after the treatment (odds ratio [OR] 2.69, 95% confidence interval [CI] 1.02–7.07), the actual marital status ([OR] 2.65, 95% CI [CI] 1.08–6.55), and the BAI score ([OR] 1.06, 95% confidence interval [CI] 1–1.12) (Table 4). Women who had changed their practice of condom use after the treatment, those without a partner and those women with higher anxiety scores had a significantly greater likelihood of feeling discomfort during colposcopy.

DISCUSSION

Women treated for SIL have a long term increased risk for recurrence of the disease, and therefore require long-term surveillance [7, 8]. They also need to take a HPV test and/or cytology, as well as colposcopy, which are the methods of follow-up. Compliance to regular follow-up is essential for timely diagnosis of recurrence. Women's attitude towards follow-up colposcopy may influence attendance of check-ups. In our cohort, 26.3% of women reported that they felt discomfort during follow-up colposcopy after cervical excisional treatment.

Our results demonstrated that women who are nulliparous, nulligravida, single or living alone represent a population, which requires additional interventions to make follow-up procedures for them more acceptable and comfortable. As we did not collect the data on mode of the delivery for women who were parous, we cannot completely rule out if the nulligravidity and nulliparity are the only factors causing discomfort during colposcopy in single women, or it is additionally influenced by the absence of vaginal deliveries in single nulliparous women. Results in relation to type of treatment also raises a question of parity influence on colposcopy discomfort registered in our study, as colposcopy was frequently unpleasant in woman treated with LLETZ, out of which 46.9% were nulliparous. This is consistent with observations that SIL treatment and colposcopy associated distress afterwards in women of childbearing age is more prolonged than initially thought and time-dependent as a result of their fertility concerns and that it persists until further reproduction is no longer an issue [13]. Moreover, literature data indicate that long-term colposcopy distress is less frequent in women who either had completed childbearing or do not intend to have children [10]. Conversely, Kola and Walsh [14] documented that women having concerns about future fertility reported greater colposcopy-related anxiety levels. Thus, although our findings regarding gravidity and parity were not statistically significant in multivariate analyses, the discomfort perceived by nulligravida and nulliparous women in our study require further evaluation.

It would be expected that women with lower BMI values feel less uncomfortable during colposcopy, which was not supported by our results. Patient's BMI in our sample was, on average, within the normal range in both the study and the control group. Therefore, the most probable reason for the difference registered in our study is the small number of obese women in our research (1.9%). In line with this explanation are the results of multivariate analysis, which failed to confirm the influence of BMI on colposcopy discomfort.

It is known that information about the sexually transmitted HPV related disease improves health related behaviors (i.e. use of condoms) [15]. The change in attitude towards condom use between study and control group registered in our cohort can be explained by psychological reasons. We hypothesize that women in the study group may have changed their attitude towards condom use probably due to their fear of HPV re-infection, as it has been described that the use of condoms provides some level of protection against HPV infections [1].

Literature reports indicate that discomfort during colposcopy is psychologically induced, and that this psychological distress is more frequent in women who have already had an abnormal colposcopy result [5, 16]. Psychological distress associated with colposcopy is particularly pronounced in women who have had previous surgical treatment [5].

Women who reported discomfort during colposcopy in our cohort exhibited higher anxiety and depression levels in comparison to women who did not report it, regardless of SIL grade. The true time duration of post-treatment adverse psychological outcomes for example anxiety and depression associated with discomfort during post-treatment colposcopy follow-up is yet to be determined. Literature reports available so far have evaluated patients on a relatively short-term basis (up to 30 months) [9]. Moreover, anxiety and depression in patients for cervical SIL have been documented to decrease with time [14]. It

has been documented that lower anxiety levels at the time of colposcopy exam are associated with higher compliance rates, which is paramount in women treated for SIL [17]. These results, in combination with our findings that follow-up colposcopy is more frequently uncomfortable in women with higher anxiety levels indicates that some women may benefit from psychological counseling aiming to reduce anxiety levels and thus prevent possible hindering follow-up adherence. Increased depression levels are also documented to be associated with non-attendance to follow-up, although this was not confirmed by the multivariate analyses in our study, which could be caused by the limited sample size [18].

Reducing anxiety levels during colposcopy decreases the feeling of discomfort during the procedure and improves adherence to follow-up [14, 19]. In line with our results, Baser et al. [19] documented the association between anxiety and discomfort during colposcopy. Additional reasons for unpleasantness documented in the literature are the fear of the associated pain, being naked during the examination and not understanding the physician's explanations, which could also be associated with anxiety [10, 20]. Measures suggested to reduce anxiety and associated feelings of discomfort include listening to music, improving communication with the health practitioner during the examination, obtaining verbal and printed information about the disease and the use of video colposcopy [14, 17, 20, 21]. All the cited authors concluded that reducing anxiety during colposcopy is also beneficial in reducing the rates of loss to follow-up.

Our findings regarding discomfort during follow-up colposcopy and its association with higher levels of anxiety and depression require further longitudinal investigation, as they could be associated with long-term default from post-treatment follow-up which is important as these women have increased risk of developing CC long-term after the treatment [7].

Despite being a single-center cross-section study, meaning that we did not obtain longitudinal data on changes of attitude towards follow-up colposcopy during the years after treatment, we managed to provide an adequate number of years of follow-up after treatment, which is the major strength of the study. Moreover, although five different physicians performed the colposcopy exams, all the women were interviewed by a single physician, in the same manner immediately after colposcopy.

There are certain limitations to this study. First, we recruited women of reproductive age who underwent follow-up in a single referral hospital. Therefore, our conclusions regarding colposcopy discomfort cannot be applied to all age groups and to untreated women. Secondly, our respondents, undergoing regular check-ups may have had a different health related behavior pattern than those women who do not undergo regular post-treatment check-ups. It is possible that at least some of the women who default from follow-up do it as they experience discomfort and anxiety during follow-up colposcopy.

CONCLUSION

Our results indicate that women of reproductive age reporting colposcopy discomfort after cervical treatment have higher levels of anxiety, which are the most frequent in these who are single, nulligravida, and nulliparous. There is a need for further research as to the interventions to reduce discomfort, anxiety, and depression in women of childbearing age that undergo colposcopy after treatment for cervical dysplasia. Such interventions aiming to reduce discomfort and psychological distress associated with the procedure could possibly lead to increased compliance rates of postoperative surveillance.

NOTE

This paper is part of the doctoral thesis of the first author with title: "Quality of life assessment in women of reproductive age treated for pathological changes in the uterine cervix," which was defended at the Faculty of Medicine, University of Belgrade, on June 13, 2018.

Conflict of interest: None declared.

REFERENCES

1. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010; 117(2 Suppl):S5–10.
2. Serrano B, Brotons M, Bosch FX, Bruni L. Epidemiology and burden of HPV-related disease. Best Pract Res Clin Obstet Gynaecol. 2018; 47:14–26.
3. Basu P, Mittal S, Bhadra-Vale D, Chami-Kharaji Y. Secondary prevention of cervical cancer. Best Pract Res Clin Obstet Gynaecol. 2018; 47:73–85.
4. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E. Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: Systematic review and meta-analysis. Lancet. 2006; 367(9509):489–98.
5. Walsh JC, Curtis R, Mylotte M. Anxiety levels in women attending a colposcopy clinic: A randomised trial of an educational intervention using video colposcopy. Patient Educ Couns. 2004; 55(2):247–51.
6. Papoutsis D, Underwood M, Parry-Smith W, Panikkar J. Early and late pregnancy outcomes in women treated with cold-coagulation versus LLETZ cervical treatment for cervical intraepithelial neoplasia; a retrospective cohort study. Arch Gynecol Obstet. 2018; 297(4):1015–25.
7. Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. Int J Cancer. 2006; 118(8):2048–55.
8. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 Updated Consensus Guidelines for the Management of Cervical Cancer Screening Test and Cancer Precursors. J Low Genit Tract Dis. 2013; 17(5 Suppl 1):S1–27.
9. O'Connor M, Gallagher P, Waller J, Martin CM, O'Leary JJJ, Sharp L, et al. Adverse psychological outcomes following colposcopy and related procedures: A systematic review. BJOG An Int J Obstet Gynaecol. 2016; 123(1):24–38.
10. O'Connor M, Waller J, Gallagher P, Martin CM, O'Leary JJ, D'Arcy T, et al. Understanding Women's Differing Experiences of Distress after Colposcopy: A Qualitative Interview Study. Women's Heal Issues. 2015; 25(5):528–34.
11. Kesić V, Sparić R, Watrowski R, Dotlić J, Stefanović R, Marić G, et al. Cross-cultural adaptation and validation of the Functional

Assessment of Chronic Illness Therapy – Cervical Dysplasia (FACIT-CD) questionnaire for Serbian women. Eur J Obstet Gynecol Reprod Biol. 2018; 226:7–14.
12. Timotijević I, Paunović VR. Instrumenti kliničke procene u psihijatriji. 3rd ed. Belgrade: Naša knjiga; 2010.
13. Juraskova I, Butow P, Sharpe L, Campion M. "What does it mean?" Uncertainty, trust and communication following treatment for pre-cancerous cervical abnormalities. Psychooncology. 2007; 16(6):525–33.
14. Kola S, Walsh JC. Patients' psychological reactions to colposcopy and LLETZ treatment for cervical intraepithelial neoplasia. Eur J Obstet Gynecol Reprod Biol. 2009; 146(1):96–9.
15. Lee Mortensen G, Adeler AL. Qualitative study of women's anxiety and information needs after a diagnosis of cervical dysplasia. Z Gesundh Wiss. 2010; 18(5):473–82.
16. Sharp L, Cotton SC, Cruickshank ME, Gray NM, Neal K, Rothnie K, et al. Long-Term Worries after Colposcopy: Which Women Are at Increased Risk? Women's Heal Issues. 2015; 25(5):517–27.
17. Galaal K, Bryant A, Deane KH, Al-Khaduri M, Lopes AD. Interventions for reducing anxiety in women undergoing colposcopy. Cochrane Database Syst Rev. 2011:CD006013.
18. Breitkopf CR, Dawson L, Grady JJ, Breitkopf DM, Nelson-Becker C, Snyder RR. Intervention to improve follow-up for abnormal Papanicolaou tests: A randomized clinical trial. Heal Psychol. 2014; 33(4):307–16.
19. Baser E, Togrul C, Ozgu E, Esercan A, Caglar M, Gungor T. Effect of Pre-Procedural State-Trait Anxiety on Pain Perception and Discomfort in Women Undergoing Colposcopy for Cervical Cytological Abnormalities. Asian Pac J Cancer Prev. 2013; 14(7):4053–6.
20. Chan YM, Lee PWH, Ng TY, Ngan HYS, Wong LC. The use of music to reduce anxiety for patients undergoing colposcopy: A randomized trial. Gynecol Oncol. 2003; 91(1):213–7.
21. Hendriksen MTJ, Van Delft KWM, Bremer GLH, Mertens HJMM. Patient satisfaction in treatment for cervical pathology. Oncol Lett. 2011; 2(3):439–43.

Дугорочни став жена у репродуктивном периоду према колпоскопском праћењу после ексцизионог лечења цервикалне дисплазије

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САЖЕТАК

Увод/Циљ Веома мало се зна о односу жена према колпоскопском праћењу после третмана грлића материце. Циљ студије је био истраживање дугорочног става жена у репродуктивном периоду према колпоскопском праћењу после ексцизије грлића материце и процена повезаности њиховог става са нивоима анксиозности и депресивности.

Методе Жене лечене ексцизијом грлића материце су интервјуисане после контролног колпоскопског прегледа. Забележене су њихове социодемографске и клиничке карактеристике. Све жене су попуниле Бекове упитнике за анскиозност и депресију.

Резултати Укупно 160 жена подељено је у студијску групу од 42 (26,3%) жене које су имале непријатност приликом контролног колпоскопског прегледа и контролну групу од 118 (73,7%) жена које нису осећале непријатност. Просечна старост у укупном узорку била је 35,3 ± 5,4 године, а медијана времена после третмана је била пет година (распон: 2–18). Жене у студијској групи су имале значајно ниже вредности *BMI*, чешће су биле нулипаре и нулигравиде, које су неудате или живе саме и значајно чешће су мењале свој однос према употреби кондома после третмана. Бекови скорови анксиозности и депресивности су били значајно већи у студијској групи. Мултиваријантна анализа је показала да су независни предиктори непријатности током контролног колпоскопског прегледа ниво анксиозности (*OR*: 1,06; 95% *CI*: 1,00–1,12), живот ван заједнице или без партнера (*OR*: 2,65; 95% *CI*: 1,08–6,55) и промена праксе употребе кондома после третмана (*OR*: 2,69; 95% *CI*: 1,02–7,07).

Закључак После ексцизијског третмана скоро трећина жена навела је непријатност током контролног колпоскопског прегледа. Ове жене су имале већи ниво анксиозности.

Кључне речи: цервикална дисплазија; конизација; праћење; колпоскопија; анксиозност; депресивност

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of antenatal maternal anxiety on the reactivity of fetal cerebral circulation to auditory stimulation, and early child development

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SUMMARY

Introduction/Objective Antenatal maternal anxiety (AMA) can have negative impact on a child's pre- and postnatal development.

The aim of the study was to examine the effect of AMA on changes in fetal blood flow through the middle cerebral artery (MCA) after acoustic stimulation, on birth outcome, and on early child development.

Methods Forty-three women in the third trimester of pregnancy and their children were enrolled into a longitudinal prospective study. The AMA (state and trait) was assessed using Spielberger's questionnaire. The MCA blood flow was assessed using Doppler ultrasound before and after the defined acoustic stimulus. The data regarding neonatal body weight, Apgar score, first step and word appearance, estimated speech–language (ESLD), sensory-motor (ESMD), and social-emotional (ESED) development at the age of three were collected.

Results Fetuses from mothers with high state and trait anxiety had slower reaction time and higher relative pulsatility index after defined auditory stimulation. There are more preterm deliveries, lower scores on ESLD, ESMD, and ESED in mothers with high State-Trait Anxiety Inventory personality trait (STAI-T) compared to low. There is a negative correlation between reaction time, relative pulsatility index, STAI-T and ESLD.

Conclusions High levels of antenatal maternal trait anxiety could have a negative effect on both fetal reactivity to sound stimulation and early child development.

Keywords: antenatal maternal anxiety; fetus; cerebral circulation; auditory stimulation; postnatal development

INTRODUCTION

There is a strong body of evidence that antenatal maternal stress and anxiety can have a negative impact on a child's pre- and postnatal development. Stress is caused by an existing, real factor or stressor. Anxiety is stress that continues to affect a person after the stressor is gone. There are two ways how antenatal maternal anxiety can manifest itself: as state or trait. The major difference between state and trait anxiety is the duration of the condition. State anxiety is short-term, while trait anxiety is a long-term, more stable tendency to experience negative emotions across many situations. Hence, trait anxiety is treated as a personality characteristic. This may be the reason why trait anxiety has a stronger impact on fetal development compared to state anxiety. Maternal stress and anxiety can lead to preterm birth and lower birth weight [1]. Moderate to high levels of antenatal maternal stress might have a negative impact on intellectual and language development in the early postnatal period [2]. In addition, studies are reporting an association between antenatal maternal anxiety and postnatal development of hyperactivity in children [3]. However, underlying mechanisms of antenatal maternal anxiety impact on fetal and early child development are not fully explored. It is known that placenta controls fetal exposure to the maternal environment, so its role could be of great importance. Cortisol appears to cross the placenta and thus may affect the fetus and disturb ongoing developmental processes. There are studies showing that high levels of anxiety could alter fetal umbilical and cerebral blood flow [4, 5]. Antenatal maternal anxiety may have a negative effect on the distribution of blood flow to the fetus. This might disturb the transportation of important nutrients to the fetal developing organs. There is a possibility to examine fetal reactivity to external defined auditory stimulus by detecting changes in fetal middle cerebral artery (MCA) blood flow change. The fetal auditory system is fully functional by the 25th week of gestation and is considered to be the major fetal information funnel. Changes in fetal cerebral circulation due to auditory stimulation are the basis of the procedure known as prenatal auditory screening (PAS) [6]. It was demonstrated previously in the group of gravid women that gestational hypertension was associated with

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significantly altered pulsatility index (Pi) values [7, 8, 9]. It was concluded that PAS measurements of Pi may be used as an indicator of psychophysiological development in the early postnatal period, while the absence of Pi changes might indicate a slow-down in fetal neurodevelopment, and particularly the maturation of the fetal auditory system.

Knowing that more expressed MCA blood flow in the PAS procedure is associated with high risk pregnancies, the aim of this preliminary study was to examine if fetal MCA blood flow changes after auditory stimulation are in correlation with antenatal maternal anxiety. We also wanted to explore the potential effect of antenatal maternal anxiety on birth outcome and early speech–language, sensory-motor, and social-emotional development. To the best of our knowledge, this is the first study that conducted a longitudinal follow-up design to examine an association between fetal reactivity to defined sound stimulation and early speech–language development due to antenatal maternal anxiety.

METHODS

Participants

Participants were recruited from a larger sample used for the longitudinal investigation of the effect of different antenatal factors on the early child development. From that sample, the sample of 72 pregnant women was selected. The general anamnestic data were collected from the participants' medical records. Participants with chronic hypertension, preeclampsia, eclampsia, diabetes, hypo- or hyperthyroidism or any other medical condition were not included in the study. Subjects used no alcoholic beverages, tobacco or any other type of psychoactive substances. None of the participants were taking any hormones during pregnancy nor any medication. None of the participants had diagnosed an anxiety disorder or any psychiatric condition. From the initial sample of 72 women, the study finally included 43 gravid women with singleton pregnancies, in gestational age between 28 and 41 weeks of pregnancy, and their babies. Table 1 presents the sample characteristics.

Table 1. Sample characteristics

n	43
Maternal age (years)	31.26 (4.18)
Education	
high (BA/BS, MA/MS, PhD)	36 (83.7%)
medium (high school diploma)	7 (16.3%)
low	0 (0%)
Marital status	
married / living with partner	43 (100%)
single	0 (0%)
Maternal monthly salary	
above average	34 (79.1%)
average	9 (20.9%)
low	0 (0%)
STAI-S score	38.21 (9.63)
low	29 (67.44%)
high	14 (32.56%)
STAI-T score	36.47 (6.96)
low	29 (67.44%)
high	14 (32.56%)
Gestational age (weeks) at testing fetal reaction to sound stimulation	35.09 (4.31)
Male fetuses (n)	17 (39.53%)
Children's chronological age at testing (in months)	39.7 (1.92)

STAI-S – state anxiety; STAI-T – trait anxiety; BA/BS – bachelor of arts/science; MA/MS – master of arts/science;
Means (standard deviations) are present for the variables maternal age, STAI-S and STAI-T score, gestational age at testing fetal reaction to sound stimulation, and children's chronological age at testing; maternal monthly salary is stratified according to the national average for 2014

Procedure

The study comprised four steps. Complete study protocol has been approved by local ethical boards, which operate in accordance with the ethical principles for medical research involving human subjects, established by Declaration of Helsinki (2013). Participants had a detailed written explanation of each of the four steps. All the participants signed their written consent prior to the study. The study was conducted at the Narodni Front Clinic for Gynecology and Obstetrics, Belgrade, Serbia, and the Institute for Experimental Phonetics and Speech Pathology, Belgrade, Serbia.

Assessment of the antenatal maternal anxiety

The first step was aimed at evaluating the anxiety level in healthy pregnant women in the third trimester using Spielberger's questionnaire form Y [10]. A total of 73 women participated in this step. The questionnaire is designed to assess anxiety as both emotional state (STAI-S) and personality trait (STAI-T). Before the study, a cutoff point of scores > 40 were selected for both state anxiety and trait anxiety [11]. The internal consistency for the state (Cronbach's $\alpha = 0.81$) and trait anxiety (Cronbach's $\alpha = 0.89$) in the sample was high. The questionnaire was given to pregnant women before the procedure of testing fetal response to defined auditory stimulation.

Testing fetal response to defined auditory stimulation

The second step was to test fetal reactivity to sound stimulation using color Doppler sonography. The first and second steps were conducted on the same day. The PAS was performed following the Protocol of Prenatal Auditory Screening as previously described by Janković-Ražnatović et al. [7], Plešinac et al. [8], and Vujović et al. [9].

Collecting data regarding the neonatal status

The third step was collecting the data regarding neonatal status of the new-born babies (a total of 47 babies participated in this step). The data about gestational age at birth (GAB: 1 – term: > 37th week of gestation, and 2 – preterm: < 37th week of gestation), body weight (BW) and Apgar score in the fifth minute were collected.

Assessment of the early child development at the age of three

The final fourth step was testing speech–language, sensory-motor, and social-emotional development of children at the age of three and collecting the data regarding early motor (appearance of the first step) and language development (appearance of the first word) at the age of one year using parental questionnaire (a total of 43 children participated in this step). To assess speech and language development (SLD), sensory-motor development (SMD) and social-emotional development (SED) of three-year-old children included in this study, we used the Scale for Evaluation of Psychophysiological Abilities of Children (SEPAC), which comprises three subscale tests (the test for assessing SLD, the test for assessing SMD, and the test for the assessment of SED) and is used in children aged 0–7 years [12, 13, 14]. All the data are collected by the method of individual testing. Depending on the achieved test scores (TS) on each subscale, expressed in percentages of the correctly done tasks, we calculated the estimated development (ED). The ED is expressed in months corrected for the chronological age (CA) using the formula: ED = CA × TS. Estimated speech–language (ESLD), sensory-motor (ESMD), and social-emotional development (ESED) are used for further analyses. The anamnestic data obtained from the children's parents in face-to-face interviews enabled the collection of data regarding the appearance of the first steps and the first words (calculated in months).

Statistical analyses

The scale variables [basal pulsatility index (PiB) reactive pulsatility index (PiR), blood flow reaction time (RT), relative pulsatility index (RePi), gestational age (GA), maternal age (MA), STAI-S, STAI-T scores, and BW) were normally distributed so parametric statistics were used. Analysis of covariance (ANCOVA) was used for the comparison of group means. We used covariates in the ANCOVA model to remove their potential contribution to the outcome variable, leaving just the adjusted group means. We employed partial correlation to measure the association between RT, RePi, STAIS, and STAI-T (as continuous variables), after controlling for the GA, MA, and fetal sex. Further, we used partial correlation to measure the association between STAI-T, RT, RePi, ESLD, ESMD, and ESED, after controlling for GA, GAB, and sex. Neonatal BW was compared between low and high anxiety groups using ANCOVA controlling for MA, fetal sex, and GAB. Apgar score was not normally distributed, so we used nonparametric Kruskal–Wallis test for the comparison of the group mean. The χ^2 test was employed to explore the differences in the distribution of GAB (in term, preterm) between low and high anxiety strata. The effect size was estimated using partial η^2 and Cohen's *d*. For each comparison, a 95% confidence interval (CI) was used. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Effect of antenatal maternal anxiety on fetal reactivity to auditory stimulation

We found no significant effect of STAI-S and STAI-T level on PiB and PiR. Figure 1 presents the mean values of RT and RePi due to low and high STAI-s and STAI-T strata.

There is a statistically significant effect of STAI-S level on RT: $F(1,38) = 15.537$, $p < 0.01$), CI 3.746–4.894, $\eta^2 = 0.285$, Cohen's d = 1.18. We also found a statistically significant effect of STAI-S level on RePi: $F(1,38) = 15.537$, $p < 0.001$), CI 13.297–22.597, $\eta^2 = 0.124$, Cohen's d = 0.73. The effect of STAI-T on RT was also notable: $F(1,38) = 9.678$, $p = 0.003$), CI 3.644–4.858, $\eta^2 = 0.199$, Cohen's d = 0.90, as well as on RePi: $F(1,38) = 5.654$, $p = 0.02$), CI 13.307–22.582], $\eta^2 = 0.127$, Cohen's d = 0.70. From Figure 1 it can be seen that both RT and RePi values are higher in the high STAI-S and STAI-T strata compared to low anxiety strata.

Effect of antenatal maternal anxiety on gestational age at birth, body weight, and Apgar score

It can be seen from Table 2 that BW was lower in babies from mothers who had high levels of both STAI-S and STAI-T anxiety. However, we found no significant effect of STAI-S and STAI-T anxiety level on BW. In addition, we found no effect of STAI-S and STAI-T anxiety level on Apgar score. We found a statistically significant difference in distribution of GAB between low and high STAI-S: $\chi^2 (1) = 10.759$, $p < 0.01$, as well as STAI-T: $\chi^2 (1) = 17.319$, $p < 0.01$. There were significantly more preterm deliveries in high compared to low STAI-S and STAI-T strata.

Effect of antenatal maternal anxiety on early speech-language, sensory-motor, and social-emotional development

We found no statistically significant effect of STAI-S on AFW, AFS, ESLD, ESMD, and ESED. In addition, we found no statistically significant effect of STAI-S on AFW and AFS. But, there was a statistically significant effect of STAI-T on ESLD: $F(1,39) = 9.526$, $p = 0.004$, $\eta^2 = 0.196$, Cohen's d = 0.96, ESMD: $F(1,39) = 8.197$, $p = 0.007$, $\eta^2 = 0.174$, Cohen's d = 0.80, and ESED: $F(1,39) = 6.004$, $p = 0.019$, $\eta^2 = 0.133$, Cohen's d = 0.69. Children from mothers who were in the high STAI-T strata achieved lower scores on each subscale of the SEPAC compared to low strata (see Table 3).

Is there an association between STAI-S, STAI-T, fetal reactivity to auditory stimulation and ESLD, ESMD, and ESED?

We found positive linear correlations between RT and STAI-S ($r(38) = 0.337$, $p = 0.033$), RT and STAI-T ($r(38) = 0.392$, $p = 0.012$), RePi and STAI-S ($r(38) = 0.367$, $p = 0.02$), and RePi and STAI-T ($r(38) = 0.356$, $p = 0.024$) (see Figure 2).

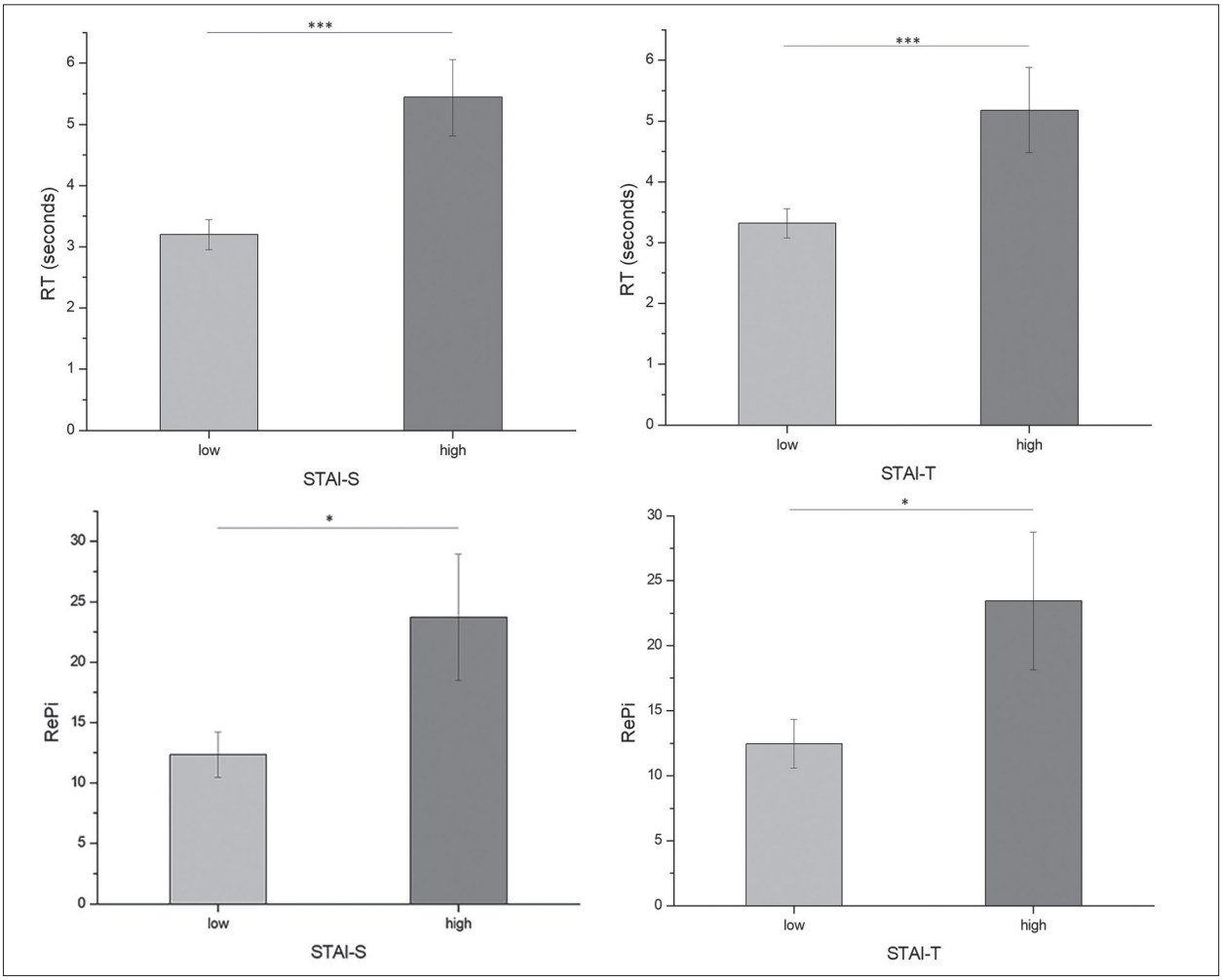

Figure 1. Fetal middle cerebral artery blood flow reaction time (RT) and relative pulsatility index (RePi) after auditory stimulation; the mean (SE) are present for low and high state (STAI-S) and trait (STAI-T) anxiety strata; *p < 0.05; ***p < 0.01.

Table 2. Birth outcome due to STAI-S and STAI-T level

STAI	Level	BW	AS	GAB-% of ptD
STAI-S	low	3,456.9 (480.09)	8.90 (0.72)	3.45% (1/29)
	high	3,103.57 (786.04)	8.64 (1.08)	13.95% (6/14)***
STAI-T	low	3,543.10 (444.96)	8.93 (0.75)	0% (0/29)
	high	2,925.00 (707.85)	8.57 (1.02)	50% (7/14)***
	total	3,341.86 (610.71)	8.81 (0.85)	16.28% (7/43)

STAI-S – state anxiety; STAI-T – trait anxiety; BW – neonatal body weight in grams; AS – Apgar score in the 5th minute after delivery; GAB – gestational age at birth; ptD – preterm delivery (< 37th week of gestation); ***p < 0.01; means (standard deviations) are present for BW and AS

Table 3. Estimated speech–language, sensory-motor, and socio-emotional development in three-year-old children due to antenatal maternal STAI-S and STAI-T level

STAI	Level	AFS	AFW	ESLD	ESMD	ESED
STAI-S	low	12.17 (2.74)	12.21 (1.26)	34.20 (2.04)	34.93 (6.35)	37.53 (4.63)
	high	13.71 (3.89)	13.21 (1.85)	32.08 (3.07)	30.05 (7.58)	35.35 (4.67)
STAI-T	low	12.17 (2.83)	12.4 (1.28)	34.31 (1.89)	35.13 (6.25)	37.87 (4.14)
	high	13.79 (3.76)	13.29 (1.77)	31.86 (3.1)***	29.63 (7.44)***	34.64 (5.18)*
	total	12.70 (3.2)	12.53 (1.53)	33.51 (2.59)	33.34 (7.07)	36.82 (4.7)

STAI-S – state anxiety; STAI-T – trait anxiety; AFS – appearance of the first steps (in months); AFW – appearance of the first words (in months); ESLD – estimated speech–language development (in months); ESMD – estimated sensory-motor development (in months); ESED – estimated socio-emotional development (in months); *p < 0.05; ***p < 0.01; means (standard deviations) are present.

There were statistically significant negative linear correlations between RT and ESLD (r(38) = -0.597, p = 0.001), RePi and ESLD (r(38) = -0.380, p = 0.016), and STAI-T and ESLD (r(38) = 0.394, p = 0.012). There were also weak negative correlations between RT and ESMD (r(38) = -0.297, p = 0.62), STAI-T and ESMD (r(38) = -0.309, p = 0.57), and RePi and ESMD (r(38) = -0.277, p = 0.84), but they did not reach the level of statistical significance (see Figure 3).

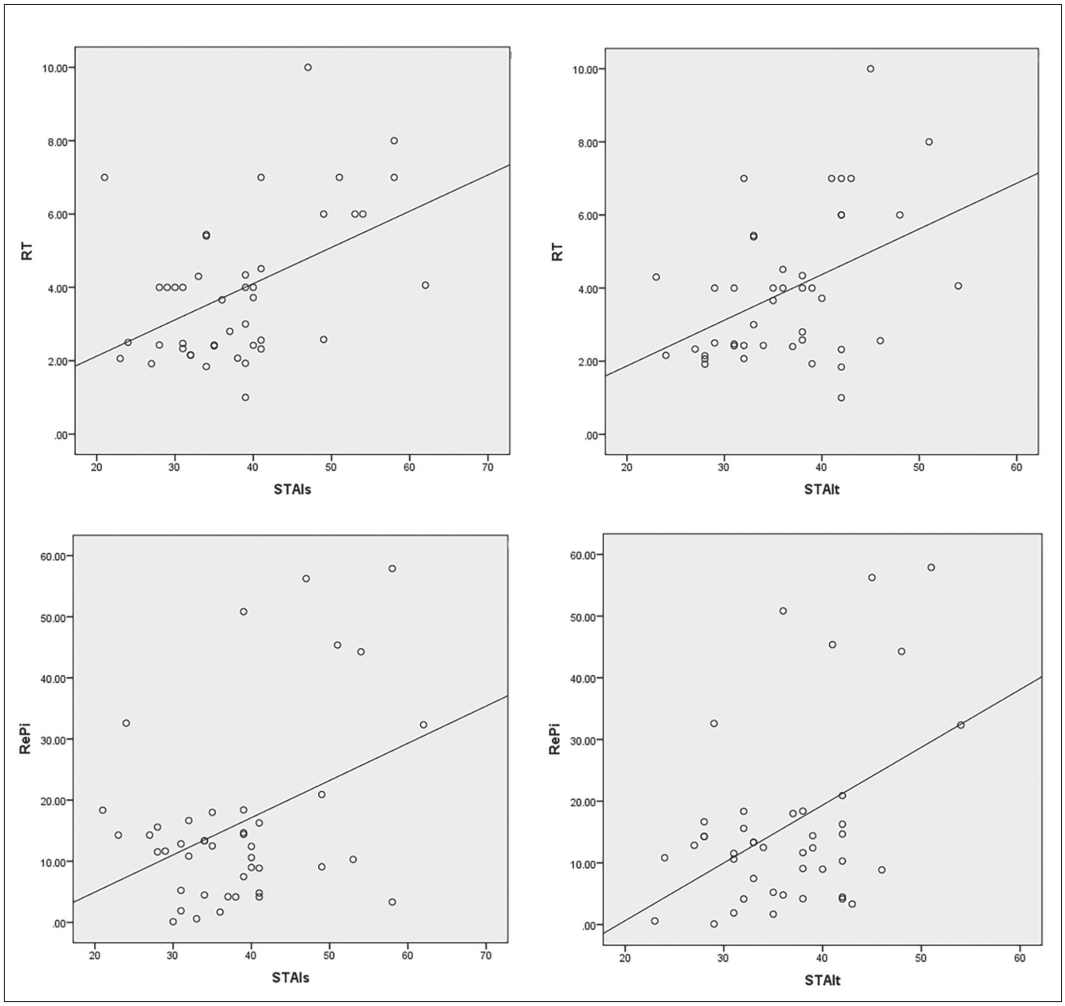

Figure 2. Correlations between fetal middle cerebral artery blood flow reaction time (RT) and relative pulsatility index (RePi) after auditory stimulation and antenatal maternal anxiety; partial correlations, controlled for maternal age, gestational age when fetal reactivity to auditory stimulation was assessed, and sex (covariates), are present in scatter plots

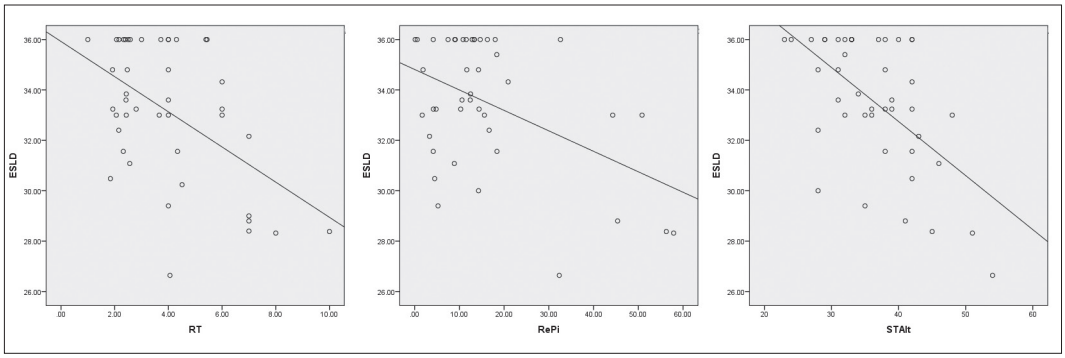

Figure 3. Correlations between estimated speech–language development and fetal middle cerebral artery blood flow reaction time (RT), relative pulsatility index (RePi), and antenatal maternal trait anxiety (STAI-T); partial correlations, controlled for gestation age at birth, gestational age when fetal reactivity to auditory stimulation was assessed, and sex (covariates), are present in scatter plots

DISCUSSION

There is a growing body of research aimed at examining how the maternal psychological state might induce long-lasting impact on fetal as well as early child development [9,15]. Lower Pi values in the MCA and higher in the umbilical artery of fetuses of mothers with high levels of trait anxiety was reported [5]. The authors concluded that there might be a change in blood distribution in favor of the brain in fetuses of highly anxious mothers. Our results are in line with this finding. We found higher cerebral blood flow through MCA after defined auditory stimulation in fetuses of mothers with high anxiety level (both state and trait) compared to low anxiety. Also, positive correlation between mean fetal artery resistance values and maternal anxiety levels are reported [4]. A recent study found an increase in

fetal MCA Pi in fetuses of mothers with both anxiety and gestational hypertension [9].

Studies are showing that the timing of testing the effect of different threatening factors on the fetal behavior is an important variable that needs to be taken into consideration. No effect of antenatal maternal anxiety on fetal spontaneous motor behavior in the first half of pregnancy was reported [16]. On the contrary, increased fetal wakefulness and increased fetal heart rate variability in fetuses of high anxious mothers in the last trimester of pregnancy have been reported [17]. We examined fetuses from the last trimester of the pregnancy. The main reason for this is the time when auditory system is mature enough so fetal reactions to defined auditory stimulation can be measured. The second reason is that the overall fetal reactivity is more expressed in second half of the pregnancy as fetus gets more mature. An increased percentage of fetal body movement and fetal heart rate variability in fetuses from highly anxious mothers has been reported [18]. This might be in line with our finding that fetal MCA RePi was higher in fetuses of mothers with high anxiety level. This might imply that reactivity is increased in fetuses of high anxious mothers. However, still remains unclear why in fetuses of high anxious mothers an increased MCA blood flow as a reaction to the external auditory stimulus was found alongside longer reaction time. In addition, we found an effect of both high state and trait anxiety on fetal MCA blood flow increase after auditory stimulation compared to low anxiety. This might be because both maternal state and trait anxiety were measured just before the experimental procedure of testing fetal reaction to defined auditory stimulation. Hence, the association between state anxiety and fetal reactivity to auditory stimulation could be biased. However, the state anxiety assessed by STAI is a measure of the current anxiety level that might have an effect on fetal cerebral circulation.

Studies have shown that negative maternal emotions during pregnancy are associated with an adverse pregnancy outcome. The effect of high antenatal anxiety on both low birth weight for gestational age and preterm delivery are the most reported results [1, 3, 9, 19]. On the contrary, no significant correlation between antenatal maternal anxiety and gestational age at birth was reported [20]. We found no statistically significant effect of both state and trait antenatal maternal anxiety on neonatal body weight. However, there were significantly more preterm deliveries in mothers with a high level of both state and trait anxiety. We found no effect of anxiety on Apgar score. There is a question why we found a significant effect of both state and trait anxiety on GAB. The answer might lie in the timing of the anxiety assessment. We assessed antenatal maternal state and trait anxiety in the third trimester. However, a recent study found that the effect of antenatal maternal anxiety on the incidence of preterm delivery remained significant regardless of the gestational period when anxiety was assessed [21].

Antenatal maternal anxiety has been associated with postnatal behavioral, emotional, and cognitive functioning [22]. Our study elicited similar results. We found a negative effect of antenatal maternal trait anxiety on speech–language, sensory-motor, and social-emotional development assessed at the age of three. It needs to be noted here that the effect size was the highest for the effect of antenatal maternal anxiety on speech–language development. Antenatal maternal psychological state had an adverse effect on an infant's speech perception and speech–language milestones and emotional regulation [23]. Not all studies found an association between prenatal exposure to maternal distress and postnatal speech–language development [24]. We found no significant effect of antenatal maternal state anxiety level on early child development at the age of three. Opposite results are reported in a recent study [25]. The authors reported a significant association between antenatal maternal state anxiety and poorer children's cognitive development at two and three years.

Limitations

This study has several limitations. First, the sample is very narrow. Hence, caution is needed in the interpretation and generalization of the results. The main idea of this study was to probe the potential effect of solely antenatal maternal anxiety on fetal reactivity to auditory stimulus, birth outcome, and postnatal development, so many factors had to be kept under control. Knowing that many factors influence child development, we had to eliminate potentially confounding factors as much as possible or to keep them under control (either methodologically during sample selection or using appropriate statistical approach). There is a recent study exploring the effect of antenatal maternal anxiety and stress on postnatal development with similar strict sample criteria [26]. The second limitation is that maternal anxiety was assessed only once during pregnancy. Although there are studies that found no association between timing of anxiety assessment, this could be misleading, especially regarding state anxiety effect on the birth outcome we found. The third limitation is that we did not measure postpartum maternal anxiety. Finally, there is always a risk that some adverse factors occurred in children during postnatal development that was not reported by parents.

CONCLUSION

This longitudinal prospective study showed a potential negative effect of high levels of antenatal maternal trait anxiety on both fetal reactivity to sound stimulation (slower reaction time and increased relative Pi) and early child speech–language and social-emotional development. In addition, there is an association between slower fetal reaction time to auditory stimulation and slower early speech–language development.

Conflict of interest: None declared.

REFERENCES

1. Pavlov M, Steiner N, Kessous R, Weintraub AY, Sheiner E. Obstetric and neonatal outcome in patients with anxiety disorders. J Matern Neonatal Med. 2014; 27(13):1339–42.
2. Laplante DP, Hart KJ, Hara MWO, Brunet A, King S. Prenatal maternal stress is associated with toddler cognitive functioning: The Iowa Flood Study. Early Hum Dev. 2018; 116:84–92.
3. Shahhosseini Z, Pourasghar M, Khalilian A, Salehi F. A Review of the Effects of Anxiety During Pregnancy on Children' s Health. Mater Sociomed. 2015; 27(3):200–2.
4. Teixeira JMA, Fisk NM, Glover V. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. BMJ. 1999; 318(7177):153–7.
5. Sjöström K, Valentin L, Thelin T, Marsal K. Maternal anxiety in late pregnancy and fetal hemodynamics. Eur J Obstet Gynecol Reprod Biol. 1997; 74(2):149–55.
6. Sovilj M, Ljubić A, Milenković V, Đoković S. Possibilities of Prenatal Examination of Reaction to Sound in Fetuses with Congenital Infections (in Serbian). In: X symposium of the prenatal medicine section of the Serbian medical society. Belgrade: Journal of Perinatal Medicine Section; 1992. p. 17–8.
7. Jankovic-Raznatovic S, Dragojevic-Dikic S, Rakic S, Nikolic B, Plesinac S, Tasic L, et al. Fetus sound stimulation: Cilia memristor effect of signal transduction. Biomed Res Int. 2014; 2014:273932.
8. Plesinac S, Jankovic S, Plecas D, Antonovic O, Adamovic T, Sovilj M. Change of Pulsatility Index of the Fetal Middle Cerebral Artery after Auditory Stimulation in No Risk Pregnancies and in Pregnancies with Gestational Hypertension. Clin Exp Hypertens. 2013; 35(8):628–31.
9. Vujović M, Sovilj M, Jeličić L, Stokić M, Plećaš D, Plešinac S, et al. Correlation between maternal anxiety, reactivity of fetal cerebral circulation to auditory stimulation, and birth outcome in normotensive and gestational hypertensive women. Dev Psychobiol. 2018; 60(1):15–29.
10. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Priručnik za Upitnik anksioznosti kao stanja i osobine ličnosti [State-Trait Anxiety Inventory (Form Y)]. Jastrebarsko: Naklada Slap; 2000.
11. Julian LJ. Measures of Anxiety. Arthritis Care. 2011; 63(0 11):1–11.
12. Adamovic T, Kosanovic R, Madic D, Ribaric-Jankes K, Sovilj M, Ethokovic S. Correlation between Balance Ability and Speech-Language Development in Children. Coll Antropol. 2015; 39(Suppl 1):11–20.
13. Rakonjac M, Cuturilo G, Stevanovic M, Jovanovic I, Jelicic-Dobrijevic L, Mijovic M, et al. Speech and language abilities of children with the familial form of 22q11.2 deletion syndrome. Genetika. 2016; 48(1):57–72.
14. Rakonjac M, Cuturilo G, Stevanovic M, Jelicic L, Subotic M, Jovanovic I, et al. Differences in speech and language abilities between children with 22q11.2 deletion syndrome and children with phenotypic features of 22q11.2 deletion syndrome but without microdeletion. Res Dev Disabil. 2016; 55:322–9.
15. DiPietro JA. Maternal stress in pregnancy: Considerations for fetal development. J Adolesc Health. 2012; 51(2 Suppl):S3–8.
16. Niederhofer H, Reiter A. Prenatal maternal stress, prenatal fetal movements and perinatal temperament factors influence behavior and school marks at the age of 6 years. Fetal Diagn Ther. 2004; 19(2):160–2.
17. Mulder EJH, Robles De Medina PG, Huizink AC, Van Den Bergh BRH, Buitelaar JK, Visser GHA. Prenatal maternal stress: Effects on pregnancy and the (unborn) child. Early Hum Dev. 2002; 70(1–2):3–14.
18. DiPietro JA, Hilton SC, Hawkins M, Costigan KA, Pressman EK. Maternal stress and affect influence fetal neurobehavioral development. Dev Psychol. 2002; 38(5):659–68.
19. Rose MS, Pana G, Premji S. Prenatal Maternal Anxiety as a Risk Factor for Preterm Birth and the Effects of Heterogeneity on this Relationship: A Systematic Review and Meta-Analysis. Biomed Res Int. 2016; 2016:8312158.
20. Littleton HL, Breitkopf CR, Berenson AB. Correlates of anxiety symptoms during pregnancy and association with perinatal outcomes: a meta-analysis. Am J Obstet Gynecol. 2007; 196(5):424–32.
21. Ding XX, Wu YL, Xu SJ, Zhu RP, Jia XM, Zhang SF, et al. Maternal anxiety during pregnancy and adverse birth outcomes: a systematic review and meta-analysis of prospective cohort studies. J Affect Disord. 2014; 159(81):103–10.
22. Plamondon A, Akbari E, Atkinson L, Steiner M, Meaney MJ, Fleming AS. Spatial working memory and attention skills are predicted by maternal stress during pregnancy. Early Hum Dev. 2015; 91(1):23–9.
23. Kaplan PS, Danko CM, Everhart KD, Diaz A, Asherin RM, Vogeli JAM, et al. Maternal depression and expressive communication in one-year-old infants. Infant Behav Dev. 2014; 37(3):398-405.
24. Ribeiro LA, Zachrisson HD, Gustavson K, Schjølberg S. Maternal distress during pregnancy and language development in preschool age: A population-based cohort study. Eur J Dev Psychol. 2016; 13(1):20–39.
25. Ibanez G, Bernard JY, Rondet C, Peyre H, Forhan A, Kaminski M, et al. Effects of antenatal maternal depression and anxiety on children's early cognitive development: A prospective Cohort study. PLoS One. 2015; 10(8):1–16.
26. Grant K, Mcmahon C, Reilly N, Austin M. Maternal sensitivity moderates the impact of prenatal anxiety disorder on infant mental development. Early Hum Dev. 2010; 86(9):551–6.

Ефекат пренаталне анксиозности трудница на реактибилност феталне церебралне циркулације после аудитивне стимулације и рани развој детета

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САЖЕТАК

Увод/Циљ Пренатална анксиозност мајке може имати негативан утицај на пренатални и постнатални развој детета. Циљ овог истраживања био је да се испита ефекат пренаталне анксиозности мајке на промену протока крви кроз медијалну церебралну артерију фетуса после аудитивне стимулације, исход порођаја и рани развој детета.

Методе Четрдесет три труднице у трећем триместру и њихова деца су учествовали у лонгитудиналној проспективној студији. Пренатална анксиозност мајке је испитивана Спилбергеровим упитником. Проток крви кроз медијалну церебралну артерију је испитан доплер ултразвуком пре и после звучне стимулације. Прикупљени су подаци о телесној тежини на рођењу, Апгаровом скору, појави првих корака и речи, процењеном говорно-језичком, сензомоторном и социоемоционалном развоју на узрасту од три године.

Резултати Фетуси трудница са високим степеном анксиозности (тренутне и оне која је црта личности) имали су спорију реакцију и веће вредности пулсатилног индекса после аудитивне стимулације. Било је више претерминских порођаја, нижих постигнућа у процењеном говорно-језичком, сензомоторном и социоемоционалном развоју деце код мајки са високим степеном анксиозности као црте личности. Утврђена је негативна корелација између времена реакције, релативног пулсатилног индекса, анксиозности као црте личности и процењеног говорно-језичког развоја.

Закључак Висок ниво пренаталне анксиозности труднице може имати негативан ефекат на феталну реактибилност, на аудитивну стимулацију и рани развој детета.

Кључне речи: пренатална анксиозност мајке; фетус; церебрална циркулација; аудитивна стимулација; постнатални развој

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Treatment of slipped capital femoral epiphysis – a comparative study during a twelve-year period

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SUMMARY

Introduction/Objective The purpose of this study was to compare two methods of treatment and to evaluate the advantages in the final outcome of transcervical fixation of the femoral head using one cannulated screw in the treatment of slipped capital femoral epiphysis.

Methods This study included 65 pediatric patients (35 boys and 30 girls), aged 6–16 years (average 11.86), during a 12-year period (2000–2012). We compared the slipping degree before and after the treatment (Southwick angle), the range of motion before and after treatment, and complication occurrence between two groups of children. The first group of children (26 patients) underwent closed reduction and cast immobilization (Group I). The other group (39 patients) was treated with transcervical fixation using one cannulated screw (Group II).

Results Comparing preoperative and postoperative Southwick angle, we found much better improvement in Group II, but without statistical significance between the two groups of patients ($p = 0.09$). Observing the range of motion of the hips before and after treatment, we found improvement in both groups of patients, especially in patients treated using transcervical fixation with cannulated screw (Group II). In complication occurrence, the patients in Group II had less complication occurrence compared to Group I ($p = 0.02$).

Conclusion The transcervical fixation using one cannulated screw has better clinical outcome and lower complication rate in relation to closed reduction and cast immobilization in the treatment of slipped capital femoral epiphysis.

Keywords: transcervical fixation; cannulated screw; closed reduction

INTRODUCTION

Slipped capital femoral epiphysis (SCFE) is the most common hip disorder in adolescence, especially in obese adolescents. It occurs in 0.2–10 per 100,000 children [1]. Also, it could be connected to endocrinological disorders, especially hypothyroidism and hyperparathyroidism [2, 3]. Etiology of SCFE is still unknown, but it is obvious that mechanical, endocrinological, and genetic factors during adolescence cause SCFE [4–11]. It has been classified according to symptom duration, weight ability, and radiographic degree of slip. SCFE can be bilateral in approximately in 20–25% of cases [12, 13].

Complications of SCFE can be early and late. Early complications are rare. Avascular necrosis (AVN) and chondrolysis are the most serious and most common late complications of SCFE. AVN is related to insufficient blood supply to the femoral neck and head after the proximal femoral epiphysis slips [4]. Epiphyseal slip severity correlates directly to late complications' occurrence [4, 7, 13].

Various procedures have been described in the treatment of SCFE: closed reduction (CR) and cast immobilization, minimal invasive surgery and percutaneous fixation or femoral osteotomies and osteosynthesis.

Prophylactic stabilization of the contralateral hip is still controversial [14, 15, 16].

The aim of this study was to compare two methods of treatment of SCFE and to evaluate the advantages of transcervical fixation of the femoral head using one cannulated screw in the final outcome.

METHODS

This retrospective study included 65 pediatric patients (35 boys and 30 girls), aged 6–16 years (average 11.86), over a 12-year period (2000–2012). Observation period was in the range of six months to 12 years (average 6.83 years). We compared the slipping degree angle before and after treatment (Southwick angle), range of motion (ROM) of the hip before and after treatment, and complications' occurrence between two groups of children [15, 16]. The first group underwent CR and cast immobilization (Group I). Group I included 26 patients (12 boys and 14 girls). The other group (Group II) was treated

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Figure 1. Anteroposterior view of slipped capital femoral epiphysis (right hip affected) before treatment

Figure 2. "Frog leg" view of slipped capital femoral epiphysis (right hip affected) before treatment

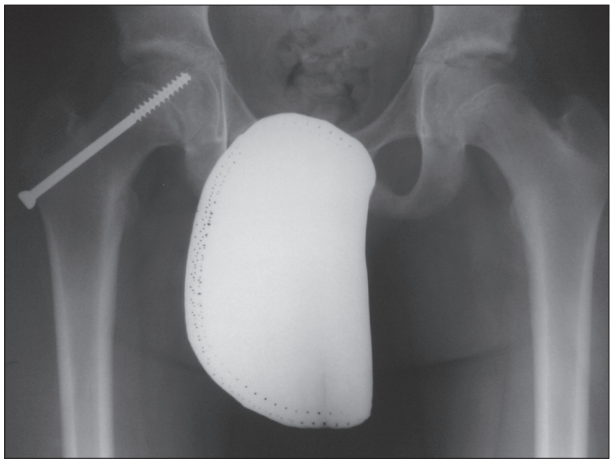

Figure 3. Anteroposterior view of slipped capital femoral epiphysis after treatment with transcervical fixation using one cannulated screw (4 mm diameter)

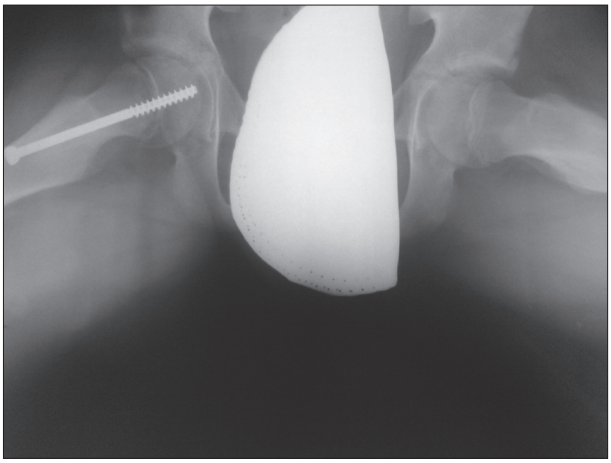

Figure 4. "Frog leg" view of slipped capital femoral epiphysis after treatment with transcervical fixation using one cannulated screw (4 mm diameter)

with percutaneous pinning using one cannulated screw. This group included 39 patients (23 boys and 16 girls). We observed various types of SCFE according to slip duration, slipping degree, and slip instability. According to SCFE types, in our study, acute slips (less than three weeks' duration) were present in 6/26 (23.08%) patients in Group I, and in 11/39 (28.21%) patients in Group II. According to weight ability, stable slips dominated in both groups – in Group I in 20/26 (76.92%) and in Group II in 33/30 (81.54%) patients. Stable slips include slips where patients can walk (with or without crutches), in contrast to unstable ones, where patients experience pain severe enough to prevents walking even with crutches. Five patients had an endocrinological contribution in SCFE – 3/26 (11.54%) in Group I and 2/39 (5.13%) in Group II. Bilateral involvement was found in 7/65 patients (10.77%).

We observed radiological and clinical outcome in patients with SCFE. The Southwick angle is the radiological parameter in SCFE we observed. It is measured bilaterally in anteroposterior and "frog leg" view, by drawing a line perpendicular to the epiphyseal line (connecting the points at anterior and posterior tips of the epiphysis) and the femoral shaft angle. The final result of the slip is obtained by subtraction from the angle of the unaffected side and is expressed in angle degrees. The clinical outcome we observed was the ROM of the hip before and after the treatment: flexion, abduction, external and internal rotation. For evaluation we used goniometer; the results are expressed in angle degrees. Also, we evaluated the complication occurrence in the observed patients. They can be early (pain, infection, malfixation) or late (avascular necrosis, chondrolysis, re-slip).

The exclusion criteria in this study were metabolic and blood vessel diseases, patients on chemo- or radiotherapy, and patients with bone dysplasia or bone tumors of the proximal femur.

Reference data was selected according to history data, clinical findings, and radiography of hips in anteroposterior and "frog leg" position.

The study was done in accord with standards of the institutional committee on ethics.

Treatment procedure and postoperative treatment

Both groups were initially treated with percutaneous traction over a period of two weeks. The traction was

applied progressively in abduction and internal rotation (with 10% of the patient's total weight on each leg). After percutaneous traction period, Group I was treated with CR and cast immobilization using maneuver according to Whitman, which means fixed position of the contralateral hip in maximal abduction (about 70°) and progressive increase of abduction (about 60°) and internal rotation (about 20°) of the affected hip and immobilization in hip spica cast [2, 4]. The cast was removed after six weeks, which was followed by physical therapy (kinesiotherapy), with progressive weight bearing (up to full-weight bearing three months after the cast removal).

The other group of patients (Group II) was treated using transcervical fixation with one cannulated screw. The patients were in the supinated position with leg in slight extension, abduction, and internal rotation. Under the C-arm fluoroscopy control, two Kirschner wires (K-wires) were inserted starting from the base of the neck to epiphysis of the proximal femur. The K-wires were used as "guides" for the cannulated screw. Before cannulated screw insertion, we performed a small 2-cm skin incision and drilling over the K-wires. After the cannulated screw was inserted, the K-wires were extracted and fluoroscopy control was done in the anteroposterior and "frog leg" position. Average cannulated screw diameter was 4 or 4.5 mm (according to the patient's age). Physical therapy started two days after the surgery, with progressive weight bearing.

Radiography was done immediately after treatment (for Group I before cast removal), three months after the treatment, and in six-month intervals up to two years after treatment. After two years, radiographic control was done annually.

Statistical interpretation

In statistical interpretation we used descriptive and analytic methods of statistical analysis. For the estimation of statistical differences between evaluated groups we used Pearson's χ^2 test, Fisher's exact test, Wilcoxon rank-sum test with continuity correction, and Mann–Whitney U test. Statistical significance was set at $p \leq 0.05$.

RESULTS

This retrospective study included 65 pediatric patients divided into two groups, depending on the method of treatment: CR and cast immobilization (Group I) or transcervical fixation using one cannulated screw (Group II). We found statistically significant differences between Group I and Group II concerning the age and body weight ($p < 0.05$) of participants, as Table 1 indicates.

Average symptom duration period for Group I was 61.77 days (range 2–180), while it was 50.72 days (range 3–180) for Group II. We found no statistical significance in symptom duration period between the two groups of patients ($p = 0.316$). Also, we found no statistical significance in side affection ($p = 0.0655$).

Table 1. Patient analysis according to sex, age, and body mass depending on the method of treatment

Parameter	Group I**	Group II***	Test
Sex			
male female	12 (46.15%) 14 (53.85%)	23 (58.97%) 16 (41.03%)	Pearson's χ^2 test $\chi^2_1 = 1.0317$; $p = 0.3097$
Age (years)			
average (SD*) median (range)	10.74 (4.27) 11 (4–18)	11.87 (4.49) 12 (3–18)	Wilcoxon rank sum test with continuity correction W = 358; $p = 0.0431$
Body mass (kg)			
average (SD*) median (range)	52.85 (13.94) 54 (17–78)	66.56 (16.89) 65 (34–100)	Wilcoxon rank sum test with continuity correction W = 277; $p = 0.0021$

*Standard deviation;
**patients treated with closed reduction and casting (Whitman method);
***patients treated using percutaneous pinning using one cannulated screw

Table 2. Type of slipped capital femoral epiphysis (SCFE) related to the method of treatment

Type of SCFE	Group I*	Group II**	Total	Pearson's χ^2 test
Acute SCFE	6 (23.08%)	11 (28.21%)	17 (26.15%)	$p = 0.64488$
Chronic SCFE	20 (76.92%)	28 (71.79%)	48 (73.85%)	
Total	26 (100%)	39 (100%)	65 (100%)	

*Patients treated with closed reduction and casting (Whitman method);
**patients treated with percutaneous pinning using one cannulated screw

Table 3. Presentation of weight ability (stable vs. unstable) in slipped capital femoral epiphysis (SCFE) depending on the method of treatment

Weight ability in SCFE	Group I*	Group II**	Total	Pearson's χ^2 test
Stable	20 (76.92%)	33 (84.62%)	53 (81.54%)	$p = 0.43358$
Unstable	6 (23.08%)	6 (15.38%)	12 (18.46%)	
Total	26 (100%)	39 (100%)	65 (100%)	

*Patients treated with closed reduction and casting (Whitman method);
**patients treated with percutaneous pinning using one cannulated screw

Table 4. Southwick angle distinction (before and after treatment) depending on the method of treatment

Treatment method	Average (SD)* distinction	Median*	Range*	Wilcoxon rank-sum test with continuity correction
Group I**	13.08 (7.63)	10	5–30	W = 629 $p = 0.09974$
Group II***	11.31 (12.4)	10	5–50	

SD – standard deviation;
*expressed in angle degrees;
**patients treated with closed reduction and casting (Whitman method);
***patients treated with percutaneous pinning using one cannulated screw

Acute and stable slips dominated in both groups of patients, but we found no statistical significance between the observed groups, as it is presented in Tables 2 and 3. Endocrinological disorders in contribution to SCFE presented no statistical significance between the two groups of patients ($p = 0.3815$).

Observing preoperative and postoperative Southwick angle, we found a better improvement in Group II, but we found no statistical significance between the two groups of patients, as Table 4 presents.

Table 5. Range of motion analysis before and after treatment of SCFE, depending of the method of treatment

Treatment method	Movement type	Before physiotherapy MV ± SD*	After physiotherapy MV ± SD*	Mann–Whitney U test (p-value)
Group I**	External rotation	38.46 ± 5.62	39.23 ± 4.84	0.696
	Internal rotation	23.46 ± 4.85	32.69 ± 3.8	< 0.001
	Flexion	106.73 ± 11.91	114.23 ± 6.43	0.036
	Abduction	29.81 ± 7	40.77 ± 3.66	< 0.001
Group II***	External rotation	37.69 ± 6.57	41.28 ± 4.25	0.018
	Internal rotation	23.33 ± 3.31	37.56 ± 3.01	< 0.001
	Flexion	107.82 ± 11.91	118.59 ± 2.8	< 0.001
	Abduction	28.72 ± 5.82	42.69 ± 2.53	< 0.001

*Mean value ± standard deviation (expressed in angle degrees);
*patients treated with closed reduction and casting (Whitman method);
**patients treated with percutaneous pinning using one cannulated screw

Table 6. Complications ratio depending on the method of treatment

Complications	Group I*	Group II**	Total	Fisher exact test
No complications	22 (84.62%)	38 (97.44%)	60 (92.31%)	p = 0.02208
With complications	4 (15.38%)	1 (2.56%)	5 (7.69%)	
Total	26 (100%)	39 (100%)	65 (100%)	

*Patients treated with closed reduction and casting (Whitman method)
**patients treated with percutaneous pinning using one cannulated screw

Table 7. Complication analysis depending on the method of treatment

Complication type	Group I* (%)	Group II** (%)	Total (%)	Fisher exact test
No complications	22 (84.62%)	38 (97.44%)	60 (92.31%)	p = 0.2208
Acute complications AVN	0 (0%) 4 (15.38%)	1 (2.56%) 0 (0%)	1 (1.54%) 4 (6.15%)	
Total	26 (100%)	39 (100%)	65 (100%)	

AVN – avascular necrosis;
*patients treated with closed reduction and casting (Whitman method);
**patients treated using percutaneous pinning using one cannulated screw

In statistical analysis of the ROM in affected hips before and after the treatment, we found an improvement in both groups of patients, but no statistical significance was found between the two groups of patients, as it is presented in Table 5.

Observing the complications' occurrence, we found significant differences in complication occurrence and severity between the two groups of patients (p = 0.022) (Table 6). In Group I we found AVN of the femoral head and neck in 4/26 patients (15.38%), and in Group II we found no AVN, but we found a re-slip in one patient (2.5%) (Table 7). In our study we found no chondrolysis among complications.

DISCUSSION

The goal in treating SCFE is early diagnosis and early treatment. We combined preoperative tractions with two methods of treatment: CR and cast immobilization and transcervical fixation using the cannulated screw.

Betz et al. [17] observed the complication occurrence (AVN and chondrolysis) in patients treated with preoperative extension, CR, and cast immobilization. The study included 32 patients (37 SCFE) over a 11-year period. They concluded that 19% of patients had chondrolysis, 3% had re-slipping of the capital femoral epiphysis, and there was no AVN recurrence. Also, Hurley et al. [18] compared re-slipping occurrence between patients treated with CR and cast immobilization and patients treated with femoral osteotomy. They concluded that 7% of patients treated with CR and cast immobilization had re-slipping versus 36% of re-slipping in patients treated with femoral osteotomy. Our study included 26 patients treated with CR and cast immobilization. The complication occurrence in our study was 15.38% (4/26 patients), presented as AVN. All of our patients affected with AVN had an unstable form with slipping of over 30°. According to our observations, we recommend an aggressive approach to unstable and severe forms of SCFE.

One of the largest comparative studies concerning treatment of SCFE was published by Kitano et al. [19]. They observed 222 patients (average age 11.8 years) with the average follow-up of 11.2 years. Preoperative slip value (according to Southwick angle) measured using X-ray films in anteroposterior and "frog like" position was 38.8° on average. They compared the treatment outcome of SCFE between patients treated with CR and cast immobilization (65 patients) and patients treated with percutaneous transcervical fixation using one cannulated screw (157 patients). Both groups of patients were treated preoperatively with percutaneous traction over a period of two weeks. According to Southwick, the most slips (43%) were below 30°, 42% of all slips were 31–60°, and 15% of the slips were over 61°. The treatment results were compared according to the Oxford score, postoperative slips and AVN occurrence. Finally, the study confirmed that unstable and acute forms of SCFE had a high risk for AVN occurrence (unstable forms 30%, acute forms 26%). Patients treated with transcervical fixation using one cannulated screw had AVN occurrence of 6%. Comparing results of this study to the results of our study, our patients had a lower preoperative slip value (23.85° for patients treated with CR and cast immobilization and 23.87° for patients treated with transcervical fixation using one cannulated screw). Also, in our study, occurrence of the mildest forms of SCFE was much higher. We found that 76.92% of the patients had a Southwick angle below 30°, compared to 43% in the study

by Kitano et al. [19]. Weight ability forms of SCFE was similar – it was 81.54% in our study, compared to 84.2% in the study by Kitano et al [19]. AVN occurrence in our study was 15.38% for patients treated with CR and cast immobilization, which is similar to the results found by Kitano et al. [19]. Concerning clinical outcome (expressed in physical findings as the ROM) before and after treatment, we found significant improvement in the ROM in both groups of patients. We prefer preoperative treatment using percutaneous traction as an important factor in clinical outcome. According to our results and the results found by Kitano et al. [19], treatment of SCFE with percutaneous traction, CR, and cast immobilization have unfavorable outcome in slips of over 30° in acute and unstable forms of slipping. Treatment of SCFE using percutaneous transcervical stabilization using one cannulated screw provides a good outcome and stability in slips below 35°. In severe slips, transcervical fixation using cannulated screw isn't as stable and becomes more vulnerable to complication occurrence.

Prophylactic stabilization of the contralateral hip is still controversial. We use it only to treat SCFE in in children younger than 10 years with endocrinological diseases.

CONCLUSION

According to our study of 65 patients with SCFE, the transcervical fixation using one cannulated screw has multiple advantages in relation to closed reduction and cast immobilization. The major effect of this method of treatment is better clinical and radiological outcome. Also, this method of treatment decreases the complication occurrence.

Conflict of interest: None declared.

REFERENCES

1. Aronsson DD, Loder RT, Breur GJ, Weinstein SL. Slipped capital femoral epiphysis: current concepts. J Am Acad Orthop Surg. 2006; 14(2):666–79.
2. Loder RT, Wittenberg B, DeSilva G. Slipped capital femoral epiphysis associated with endocrine disorders. J Pediatr Orthop 1996; 15(3):349–56.
3. Paley D. Growth Plate Considerations. Berlin: Springer-Verlag; 2002. p. 695–716.
4. Slavkovic N, Vuksinovic Z, Slavkovic S. Factors influencing the development of avascular necrosis in non-operative treatment of the acute slipped capital femoral epiphysis. Srp Arh Celok Lek. 2007; 135(1–2):54–60.
5. Cohen MS, Gelberman RH, Griffin PP, Kasser JR, Emans JB, Millis MB. Slipped capital femoral epiphysis: Assessment of epiphyseal displacement and angulation. J Pediatr Orthop. 1986; 6(3):259–64.
6. Gelberman RH, Cohen MS, Shaw BA, Kasser JR, Griffin PP, Wilkinson RH. The association of femoral retroversion with slipped capital femoral epiphysis. J Bone Joint Surg Am. 1986; 68(7):1000–7.
7. Vukasinovic Z, Slavkovic N, Slavkovic S. Complications of slipped capital femoral epiphysis. Srp Arh Celok Lek. 2007; 135(1–2):105–10
8. Slavkovic N, Vukasinovic Z. Slipped capital femoral epiphysis: a modern treatment protocol. Srp Arh Celok Lek. 2009; 137(9–10):562–6.
9. Blethen SL, Rundle AC. Slipped capital femoral epiphysis in children treated with growth hormone. A summary of the National Cooperative Growth Study experience. Horm Res. 1996; 46(3):113–6.
10. Allen CP, Calvert PT. Simultaneous slipped upper femoral epiphysis in identical twins. J Bone Joint Surg Br. 1990; 72(5):928–9.
11. Peck D. Slipped capital femoral epiphysis: diagnosis and management. Am Fam Physician. 2010; 82(3):258–62.
12. Hägglund G, Hansson LI, Ordeberg G. Epidemiology of slipped capital femoral epiphysis in southern Sweden. Clin Orthop Relat Res. 1984; 191:82–94.
13. Hansson G, Jerre R, Sanders SM, Wallin J. Radiographic assessment of coxarthrosis following slipped capital femoral epiphysis: A 32-year follow-up study of 151 hips. Acta Radiol. 1993; 34(2):117–23.
14. Seller K, Raab P, Wild A, Krauspe R. Risk-benefit analysis of prophylactic pinning in slipped capital femoral epiphysis. J Pediatr Orthop B. 2001; 10(3):192–6.
15. Jerre R, Billing L, Hansson G, Karlsson J, Wallin J. Bilaterality in slipped capital femoral epiphysis: Importance of a reliable radiographic method. J Pediatr Orthop B. 1996; 5(2):80–4.
16. Seller K, Wild A, Westhoff B, Raab P, Krauspe R. Radiological evaluation of unstable (acute) slipped capital femoral epiphysis treated by pinning with Kirschner wires. J Pediatr Orthop B. 2006; 15(5):328–34.
17. Betz RR, Steel HH, Emper WD, Huss GK, Clancy M. Treatment of slipped capital femoral epiphysis. Spica-cast immobilization. J Bone Joint Surg Am. 1990; 72(4):587–600.
18. Hurley JM, Betz RR, Loder RT, Davidson RS, Alburger PD, Steel HH. Slipped capital femoral epiphysis. The prevalence of late contralateral slip. J Bone Joint Surg Am. 1996; 78(2):226–30.
19. Kitano T, Nakagawa K, Wada M, Moriyama M. Closed reduction of slipped capital femoral epiphysis: high-risk factor for avascular necrosis. J Pediatr Orthop B. 2015; 24(4):281–5.

Лечење склизнућа главице бутне кости – упоредна студија у периоду од дванаест година

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САЖЕТАК

Увод/Циљ Циљ ове студије је поређење две методе лечења и процена предности резултата лечења трансцервикалном фиксацијом главе бутне кости употребом једног канулираног завртња у лечењу склизнућа главе бутне кости.

Методе У студију је укључено 65 педијатријских болесника (35 дечака и 30 девојчица), узраста од 6 до 16 година (просечна вредност 11,86), током 12-годишњег периода (од 2000. до 2012. године). Упоређивали смо степен склизнућа пре и после спроведеног лечења (Саутвиков угао), обим покрета пре и после спроведеног лечења и учесталост компликација између две групе болесника. Прва група (26 болесника) лечена је затвореном репозицијом и имобилизацијом гипсаним завојем (I група), а друга група (39 болесника) била је лечена перкутаном фиксацијом једним канулираним завртњем (II група).

Резултати На основу поређења преоперативних и постоперативних вредности Саутвиковог угла, болесници друге групе су имали бољи радиографски резултат у односу на болеснике из прве групе, али без статистички значајне разлике ($p = 0,09$). Посматрајући обим покрета кукова пре и после интервенције, забележено је значајно побољшање у обе групе болесника, посебно код болесника лечених трансцервикалном фиксацијом једним канулираним завртњем (група II). Посматрајући учесталост компликација, болесници друге групе су имали мањи број компликација ($p = 0,02$) у односу на болеснике прве групе.

Закључак Метода трансцервикалне фиксације главе бутне кости је дала бољи клинички резултат и мањи број компликација у односу на методу ортопедске репозиције и имобилизације гипсаним завојем у лечењу болесника са склизнућем главе бутне кости.

Кључне речи: трансцервикална фиксација; канулирани завртањ; затворена репозиција

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Ocular biometric changes after trabeculectomy

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SUMMARY

Introduction/Objective Trabeculectomy is a conventional filtration procedure in surgical glaucoma treatment. Even after successful trabeculectomy, the patient's visual acuity can be reduced. Studies (1991) showed that changes in visual acuity occur due to changes of corneal curvature and anterior chamber depth. Anterior chamber depth change for 1 mm results in about 2 diopters change in refractive sphere. Simultaneous with anterior segment changes, anti-glaucoma surgery effect can also be manifested in posterior segment of the eye: choroidal thickness, axial length and the ocular perfusion. Axial length reduction after trabeculectomy was supposed according to biometry, more pronounced if intraocular pressure is higher preoperatively, or in the first postoperative week with spontaneous recovery to preoperative values one year after surgery. A study was conducted at the Clinic for Eye Diseases in Niš to determine the difference in pre/post-operative values of biometry on 60 patients with glaucoma.

Methods In this study we used retrospective-prospective biometric analysis in patients with open-angle glaucoma.

Results Anterior chamber depth was significantly different during the observed period, for 1.1 mm in first week ($p < 0.0001$) in the whole group and glaucoma type. The mean axial length varies considerably during the observed period, shorter for 0.39 mm in first week ($p < 0.05$).

Conclusion By analyzing biometric parameters, a postoperative difference of biometry with spontaneous recovery was determined. There is a difference in postoperative visual acuity of patients compared to preoperative, with spontaneous recovery at the end of the follow-up.

Keywords: glaucoma; trabeculectomy; ocular biometry; anterior chamber depth; axial length

INTRODUCTION

Trabeculectomy was developed in 1968. (Cairns) and still remains the most frequent filtration procedure in surgical glaucoma treatment that bypasses the aqueous outflow by creating a corneo-scleral fistula that leads in forming subconjunctival bleb. By using potential wound modulators (5-fluorouracil, Mitomycin-C) prolonged hypotensive effect could be achieved and yearly success rate for trabeculectomy increased to 70–80%. One of the greatest disappointments of glaucoma filtering surgery is suboptimal postoperative visual acuity. Even after successful procedure, it can be reduced for several lines at Snellen chart. The primary reason for poor postoperative visual acuity after filtering surgery could be astigmatism, hypotony, inflammation, or hyphema. Trabeculectomy leads to a large decrease in intraocular pressure (IOP) especially in early postoperative period. Potential complications include hypotony, choroidal effusion, hemorrhage, hypotonus maculopathy, corneal decompensation, and cataract [1]. After treating all medical reasons, refractive factors could remain (corneal curvature and ocular biometric changes), that are dynamic and changeable in postoperative period.

Biometry, according to early definition, is the method of applying mathematics and measurement to biology. The term was originally used by Whewell initially in the 1800s [2]. Ultrasound biometry is the measurement of various eye dimensions, its components, and their interrelationships. Ultrasound (echography, B-scan) uses high frequency sound waves (10 MHz) to produce images of the internal eye structures. Ultrasound biometry (A-scan) utilizes an ultrasound device for eye measurement: axial length (AL), anterior chamber depth (ACD), crystalline lens thickness, diagnosing and measuring masses in the eyes. As an invasive procedure, it requires direct ultrasound probe contact with the cornea by trained examiner to avoid errors due to excessive compression on cornea [3]. AL is the distance from anterior corneal surface to the retinal pigment epithelium. It can be done using optical or ultrasound methods, which can further be done by direct contact or immersion [4].

Ocular biometric changes after trabeculectomy with refractive implications are in AL and ACD. AL decreases due to improvement of ocular blood flow and increased choroidal thickness (CT) as dynamic parameter that is influenced by oscillations in IOP. Large decrease in IOP after trabeculectomy during the early

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postoperative period, may lead to significant CT changes and thickening because of the improved ocular perfusion. Until recently, no imaging modality could get accurate in vivo CT measurements. Enhanced depth imaging-optical coherence tomography uses low signal strength to achieve greater depth than spectral domain optical coherence tomography [5, 6].

Another factor affecting postoperative visual acuity is ACD. This was due to refraction shift secondary to depth changes, 1 mm change in ACD results in approximately 2 diopter change in refractive sphere (DSph). Visual acuity starts to return to pre-operative by the third post-operative week and usually recovers fully within six weeks. This alteration is caused by iris-lens plane movement partially. If cycloplegic drug was not applied postoperatively, anterior chamber tends to shallow in the post-operative period and maximal shallowing occurs by the fifth day with spontaneous reformation after two weeks [7, 8, 9].

It is stated that trabeculectomy can cause ACD changes and even small AL change could lead to unsatisfactory visual acuity and significant error in IOL power calculation and refractive prediction in cataract surgery [6]. Aim of this work is to determine presumed difference between pre/postoperative biometrics findings in glaucoma patients.

METHODS

This retrospective-prospective clinical investigation included 60 phakic eyes, of 60 operated patients with Open Angle Glaucoma (OAG) in two groups: 42 Primary Open Angle Glaucoma (POAG) patients and 18 Exfoliative Glaucoma (XFG) patients, to determine postoperative changes of biometry at the seventh, 30th, 60th day after standard trabeculectomy without antimetabolites. The study was performed from March 2015 to March 2016 at the Ophthalmology Clinic, Clinical Center of Niš, according to the Ethical Committee principles of the Helsinki Declaration, and written consent was obtained from all the participants. Each patient underwent pre/postoperative measurements of same parameters: visual acuity (Snellen chart), slit lamp examination, Goldmann applanation tonometry ACD, AL (Tomey Ultrasonic A/B scanner and biometer UD6000).

Although there are two modalities of A-scan ultrasound biometry available, contact and immersion, in this investigation contact A-scan biometry measurements were obtained because it is faster and simple to perform. After topical anesthesia of ocular surface (tetracaine hydrochloride 0.5%) ultrasound probe was placed on the vertex of cornea carefully, to avoid corneal indentation and off-axis measurements. All measurements were obtained by one ophthalmologist in aim to minimize inter-observer variability of results.

A special crystal embedded in a probe oscillates to generate a high-frequency ultrasound wave that penetrates the eye. These results are in one-dimensional time-amplitude representation of echoes received along the beam path. The height of the spikes is indicative of the strength for the specific eye tissue. The distance between the echo spikes recorded on the oscilloscope screen provides an indirect measurement of tissue such as ACD or AL of the eye and displayed in millimeters on the screen.

Obtained results were analyzed by statistical analysis and application of software SPSS 18 statistical package.

RESULTS

Effect of trabeculectomy, in surgically treated OAG patients is shown through IOP values and visual acuity changes in Table1 and 2. The mean IOP value shows a significant difference (Friedman test χ^2; $p < 0.0001$) in subjects compared to preoperative values. The average preoperative IOP of 32.5 ± 8.64 mmHg decreases in the next two months to a value 15.93 ± 3.46 mmHg at the end of a two-month monitoring interval. Similar values and fluctuations of IOP were in both groups of operated glaucoma patients (POAG, XFG) with discrete higher values in POAG group, from 33.38 ± 9.08 mmHg preoperatively to16.36 ± 3.51 mmHg two months later in comparison to XFG group of respondents with 30.44 ± 7.35 mmHg before surgical intervention, lowered to 14.94 ± 3.23 mmHg two months after the operation (Table 1).

Table 1. Intraocular pressure values (mmHg) in operated Open Angle Glaucoma patients in the time interval

Intraocular pressure in days	Intraocular pressure values in glaucoma type			
	Glaucoma type	Number of patients	Average intraocular pressure	Standard deviation
Intraocular pressure day 0	POAG	42	33.38	9.08
	XFG	18	30.44	7.35
	Total	60	32.50	8.64
Intraocular pressure day 7	POAG	42	14.90	4.39
	XFG	18	14.28	5.40
	Total	60	14.72	4.68
Intraocular pressure day 30	POAG	42	15.90	4.07
	XFG	18	16.44	4.85
	Total	60	16.07	4.28
Intraocular pressure day 60	POAG	42	16.36	3.51
	XFG	18	14.94	3.23
	Total	60	15.93	3.46

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

The visual acuity shows statistically significant difference in operated glaucoma patients in two-month interval (Friedman Test χ^2; $p < 0.0001$). From 0.4 ± 0.35 decreases for two lines on Snellen chart on the seventh postoperative day (0.22 ± 0.22), then improves for one line (30th postoperative day) and returns almost to the preoperative visual acuity in the next two months (0.37 ± 0.32). Similar visual acuity was in both glaucoma groups of patients (Table 2).

Among OAG group there was 28 (46.67%) female respondents with the mean age of 72.2 ± 7.92 years and the total number of men was 32 (53.33%) with the mean age 65.4 ± 11.56 years. Females are significantly older ($t = 2.626$; $p = 0.011$) (Table 3).

Of all patients 42 (70%) suffered from POAG with the mean age of 66.7 ± 11.1 years and the remaining 18 (30%) were patients with XFG type of glaucoma with the mean

Table 2. Visual acuity in glaucoma type (POAG, XFG) in time interval

Visual acuity days	Visual acuity in glaucoma type			
	Glaucoma type	Number of patients	Average visual acuity	Standard deviation
Visual acuity day 0	POAG XFG Total	42 18 60	0.41 0.37 0.4	0.35 0.36 0.35
Visual acuity day 7	POAG XFG Total	42 18 60	0.22 0.22 0.22	0.22 0.23 0.22
Visual acuity day 30	POAG XFG Total	42 18 60	0.28 0.29 0.28	0.25 0.3 0.26
Visual acuity day 60	POAG XFG Total	42 18 60	0.37 0.36 0.37	0,32 0.33 0.32

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

Table 3. Sex structure

Sex	Years of life			
	Number of patients	Percent of patients	Mean age	Standard deviation
Female	28	46.67%	72.2	7.92
Male	32	53.33%	65.4	11.56
Total	60	100%	68.6	10.52

Table 4. Glaucoma type according to sex and years of life

Sex	Glaucoma type		
	POAG	XFG	Total
Female %	21 75%	7 25%	28 100%
Male %	21 65.63%	11 34.38%	32 100%
Total %	42 70%	18 30%	60 100%
Age Standard deviation	66.67 11.11	73.06 7.49	68.58 10.52

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

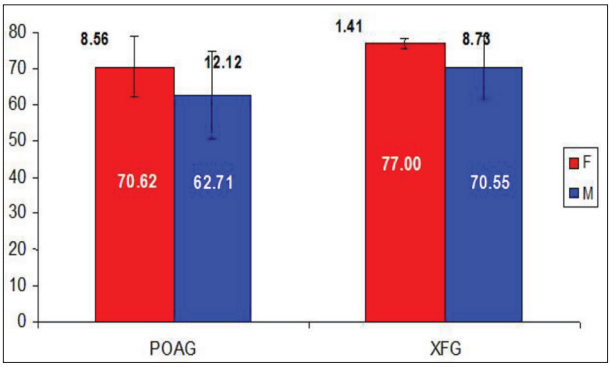

Figure 1. Age structure of respondents by sex in both glaucoma types

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

Table 5. Time distribution of anterior chamber depth

Anterior chamber depth (mm)	Anterior chamber depth in glaucoma type			
	Glaucoma type	Number of patients	Average anterior chamber depth	Standard deviation
Anterior chamber depth day 0	POAG XFG Total	42 18 60	2.73 2.94 2.79	0.56 0.81 0.65
Anterior chamber depth day 7	POAG XFG Total	42 18 60	3.85 3.99 3.89	1.45 1.37 1.42
Anterior chamber depth day 30	POAG XFG Total	42 18 60	3.59 3.59 3.59	1.06 1.64 1.25
Anterior chamber depth day 60	POAG XFG Total	42 18 60	3 3.28 3.08	0.84 1.37 1.02

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

Table 6. Time distribution of axial length change

Axial length (mm)	Axial length values in glaucoma type			
	Glaucoma type	Number of patients	Average AL	Standard deviation
Axial length day 0	POAG XFG Total	42 18 60	23.49 23.24 23.41	1.4 0.85 1.26
Axial length day 7	POAG XFG Total	42 18 60	23.07 22.88 23.02	1.37 1.03 1.27
Axial length day 30	POAG XFG Total	42 18 60	23.14 22.98 23.09	1.26 0.9 1.16
Axial length day 60	POAG XFG Total	42 18 60	23.22 23.69 23.36	1.25 2.9 1.89

POAG – primary open angle glaucoma; XFG – exfoliative glaucoma

age of 73.1 ± 7.5 years. Patients with XFG type are significantly older (t = 2.195; p < 0.05). The mean age of the operated group of patients was 68.58 ± 10.5 years (Table 4).

Following graph presents age structure of respondents by sex in both glaucoma types (POAG and XFG) (Figure 1). Females in XFG group are the oldest, 77 years on average. Male respondents in XFG group are younger (70.55 years) than females. In POAG group, females are older (70.62 years) than male (62.71 years).

Upcoming results illustrate implications of anterior segment anti-glaucoma surgical approach to biometric parameters of the eye.

ACD varies considerably during the two-month period (Friedman test χ^2; p < 0.0001) in the whole group and according to glaucoma type (POAG; XFG). From an average preoperative value of 2.79 ± 0.65 mm, its value increases mostly on the seventh day (3.89 ± 1.42 mm), then decreases on the 30th day (3.59 ± 1.25 mm) and further reduced at the end of the following period to 3.08 ± 1.02 mm, near to preoperative values. Similar values of ACD were in both glaucoma types during the two-month time interval (Table 5).

The mean AL varies considerably during the observed monitoring period (Greenhouse-Geisser; p < 0.05). Preoperatively, the mean value was 23.41 ± 1.26 mm for the whole group, and its value decreased by a small but significant amount, reaching 23.02 ± 1.27 mm on the seventh day and 23.09 ± 1.16 mm on the 30th day, and then increased at the end of the second month to the approximate preoperative value of 23.36 ± 1.89 mm. A week after operation, AL became shorter for 0.39 mm. Similar measurements were found in both glaucoma types. In POAG it become shorter for 0.42 mm a week later and in XFG was shorter 0.36 mm the first week after operation (Table 6).

The dynamic relationship between change in IOP and the state of sclera and choroid manifested and showed as

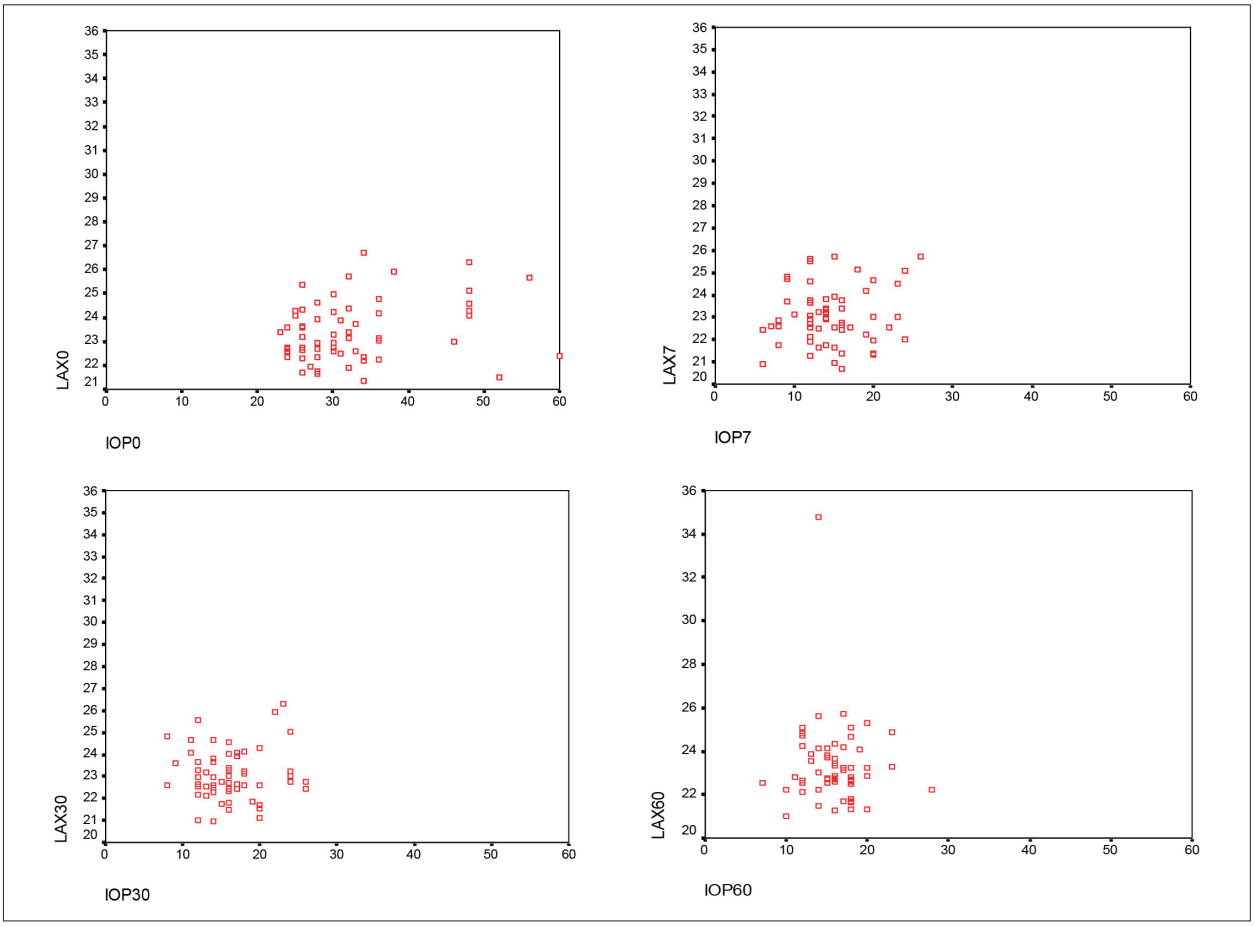

Figure 2. Intraocular pressure and axial length relationship through time

AL parameter is presented through time and confirmed by AL sequential measurements in postoperative period. IOP was obviously lowered after operation providing estimation of the magnitude of AL decrease with IOP lowering (Figure 2).

DISCUSSION

Trabeculectomy is commonly performed in patients with open angle glaucoma when medical therapy fails to control IOP. This procedure appears to be the best surgical method for preventing progressive optic disc damage [5]. Results of Early Manifest Glaucoma Trial indicate that IOP reduction of 25% from baseline reduces disease progression risk by 50%. For each mmHg IOP reduction, progression risk decreases for 10%. It is accepted that normal IOP was estimated based on the measurement in more than thousand people as the mean IOP ± 2(3) standard deviation. Normal IOP in healthy adult population is 15–16 mmHg ± 3 mmHg, in the interval 10–21 mmHg [10]. In this study mean preoperative IOP 32.5 ± 8.64 mmHg was most decreased on the seventh postoperative day (14.72 ± 4.68 mmHg), and then slightly elevated in next two months, to a value of 15.93 ± 3.46 mmHg at the end of monitoring. This fluctuation was within European Glaucoma Society Guidelines recommendations. Observing average reduction of 16.57 ± 5.18 mmHg from baseline, we achieved reduction of 50.98% ($p < 0.0001$). Equal efficiency was achieved in both glaucoma types (POAG, XFG).

Based on the International Glaucoma Association recommendation for results based on average statistical estimates of surgical success, trabeculectomy outcome was classified as failure (target IOP not achieved), complete failure (loss of light perception), complete success (IOP achieved without therapy) and qualified success (IOP achieved with topical therapy). Analyzing IOP in operated patients, as an indicator of surgical efficacy, we concluded complete success in our group for two months without topical anti-glaucoma medication.

Detailed assessment of the effect of the surgical procedure requires monitoring over a longer period, at least a year after surgery with the aim of prognostic conclusions according to Scandinavian Trabeculectomy Study (POAG, XFG). They reported a total success of 82% one year after surgery, 70% after two years, 64% after three, and 52% after four years of follow-up with a better treatment rate of POAG than of XFG [11]. The first published national experience of surgical success in POAG in Britain was carried out in 2000 under patronage of the National Health Service and Public Health Department, presented 92% success rate in one-year follow-up [12].

However, despite the fact that IOP is the only parameter in evaluation of antiglaucoma surgery effectiveness,

we should not the influence of glaucoma surgery on the refractive outcome and visual acuity due to the changes in the anterior and posterior eye segment. Actual ophthalmic trends introduce the term refractive glaucoma surgery. As in cataract surgery, patients expectations and demands have been increased in terms of achieving a good refractive outcome, faster postoperative recovery, less complications, without compromising the quality of life. In the past, surgeons focused mainly on effective IOP control. Today, patients rarely accept blurred vision due to astigmatism, hypotony, or discomfort caused by filtration bleb. Modern glaucoma surgeons deal with the modification of surgical techniques to achieve more diffuse pads, minimal astigmatism, and wound healing modulation with faster recovery.

In this study, the best-corrected pre/postoperative visual acuity was tested at Snellen chart over time (seventh, 30th, 60th day). World Glaucoma Association recommends that two methods can be used in the estimation of visual acuity: ETDRS, and Snellen charts, that is used in our routine clinical practice [13].

The average preoperative visual acuity in both glaucoma types was approximately four lines at Snellen chart. On the seventh postoperative day, there was a significant visual acuity decrease in both glaucoma types to 0.2 according to the Snellen chart ($p < 0.0001$). On the 30th day visual acuity was improved for one line (0.3 Snellen) and in 60 days in both groups returned to approximate preoperative acuity of 0.37 (four lines at Snellen chart). To prevent hypotonia, inflammation, shallow anterior chamber and other complications, topical mydriatic (cycloplegic) solution Homatropine 2% was administered two weeks after surgery. This could explain visual acuity reduction in the first postoperative week due to wide pupils and cycloplegia. There was no bleeding or choroidal effusion as complications that could affect visual acuity.

By analyzing demographic characteristic in OAG, a uniformed relationship between sex (46.67% female *vs*. 53.33% male) and years of life was observed. XFG is a rare type of glaucoma, more common in elderly people, that corresponds with results in our group. Out of all the patients, 70% of them presented with POAG at the average age of 66.67 years, and 30% of the patients presented with XFG at the average age of 73.06 years. World studies report that XFG prevalence shows geographic, racial, ethnic variations. Australia, Sweden, Finland, Norway, Russia, Greece, Turkey, Iran, Saudi Arabia, Tunisia reported an XFG incidence of 13–21% in people over 60. Lower rate than 6% in patients over 70 is recorded in Germany, Britain, Japan, Austria, Switzerland, and Denmark. The prevalence increases with age from 2.8/100,000 at 50, to 205.7/100,000 in 79 years [14, 15, 16]. Respondents' age has a positive correlation with glaucoma type (XFG). According to our results, XFG was observed at a significantly older age, which was 77 years for females and 70.55 for males, in contrast to 70.62 years for females and 62.71 years for males in POAG. In Serbia, half of all glaucoma occurs in people over 65, while the other half is the working age population between 40–65 years [10]. According to the results of multicenter clinical study in Russia, blindness occurs at the age of 75, therefore scientists point out the importance of surgical therapy on time, despite the transient change of visual acuity.

In the 1960s, the first literature review of reduced visual acuity after trabeculectomy was found in Watson's work, as consequence of iris position change. According to his observations, it lasted for six weeks. Detailed analysis by Cunliffe et al. [7] implies reduction of uncorrected and best-corrected visual acuity three weeks after surgery in subjects without cycloplegic. Although surgery benefits in glaucoma control, studies have indicated that it affects corneal curvature, ocular biometry and leads to visual acuity deterioration [17, 18]. The study by Cunliffe et al. [7] showed 94% of eyes worsen with uncorrected acuity a week following the surgery. This was due to a "myopic shift" in refraction, secondary to anterior chamber depth changes, which became shallow because they did not receive cycloplegic, so ciliary body rotated forward, accommodated, and moved to myopic refraction. Only 1 mm change in anterior chamber depth results in 2 diopter change in refractive sphere. Visual acuity starts to return to pre-operative levels in variable period, so to determine changes in our patients; we continued research of obtained parameters ACD and AL.

ACD is an important factor in visual acuity change in early postoperative period because it decreases on the fifth day with spontaneous reforming until the second week, if the cycloplegic drops were not applied. Raitta tried to reform anterior chamber by viscoelastic Na-hyaluronate in aim to prevent shallowing, because the rotation and movement of ciliary body are progressing with the spasm, which increases "myopic shift" [7]. Our postoperative protocol includes homatropine solution for two weeks after surgery to avoid athalamia. This is in aim of achieving better surgical outcome, preservation of filtration bleb integrity which could be bad especially in presence of iridocorneal contact [7, 19]. Shallower AC preoperatively is common finding in XFG, indicates zonular instability and increased incidence of postoperative complications.

Our biometric parameters analysis showed that average ACD varies during the monitoring period, from preoperative 2.79 ± 0.65 mm, its value increases the most at the seventh day (3.89 ± 1.42) then decreases on the 30th (3.59 ± 1.25) and on the 60th day (3.08 ± 1.02) almost to preoperative level ($p < 0.0001$). Deepening of AC was due to cycloplegic application (about 15 days) according to protocol thus providing stable chamber at the end of follow-up.

The most systematic review of all relevant articles in biometric changes published on PubMed from 1989–2016 was analyzed by Alvani et al. [6] who selected 25 comparable studies including 690 individuals which met similar inclusion criteria. All studies revealed ACD reduction immediately after surgery, which gradually deepened and reached its preoperative levels on day 14 in 87–91% of participants. ACD reduction was not significant after that period in majority of cases. ACD change of small amount in short period independently from measuring method: A-scan, ultrasound biometry, pachymetry, or optical biometry, was also noticed by Husain et al. [20] and Chen (2014) [21, 22].

Other ocular biometric change after trabeculectomy is axial length (AL) decrease due to improvement of ocular blood flow [5]. As choroidal thickness is dynamic parameter influenced by IOP oscillations, large IOP decrease might lead to significant CT/AL changes. Alvani et al. [21] analyzed eight studies that evaluated AL, all of them reported significant AL reduction postoperatively at all follow up points for 0.1–0.9 mm that became stable after three months. These studies were different in terms of follow up period, intra-operative antimetabolites application, method of AL measurement [6, 16, 20, 23]. Nemeth and Horoczi [24] noticed AL reduction four days after trabeculectomy for the first time. Cashwell and Martin [25] examined records of 62 patients and measured AL before and after surgery by ultrasound biometry, then reported the mean AL reduction of 0.423 ± 0.61 mm that lasts for 22.5 months. Studies of this kind differ in measurement method and antimetabolites application. A high percent of patients with antimetabolite had lower IOP and frequently experienced greater amounts of biometric changes [23–26]. For first time, Kook et al. [27] reported a significant AL reduction after trabeculectomy with mitomycin C (MMC), for 0.83 ± 1 mm at the follow up period of three months. Notice that different AL measurement methods explain disparity between studies results. Ultrasonic methods led to more pronounced AL reduction than noncontact techniques [20].

Our study was performed without MMC and showed that preoperative value of 23.41 ± 1.26 mm AL decreased at the seventh day (23.02 ± 1.27 mm) and 30th (23.09 ± 1.16 mm), then raised on the 60th, almost to preoperative (23.36 ± 1.89 mm) ($p = 0.043$; $p < 0.05$). This AL reduction for 0.39 ± 1.27 mm is very similar to Cashwell and Martin's results. Most studies confirmed AL changes causes and showed: high preoperative IOP, low postoperative IOP, young age, antimetabolite application, myopic refraction, surgical complications (choroidal detachment, hypotony maculopathy) were significantly associated with prolonged AL reduction [23, 27].

The direct relationship between IOP reduction, CT increase and AL reduction has been demonstrated in several studies. In a study by Husain et al. [20], each 1 mmHg decrease in IOP led to a 0.01 mm AL reduction in POAG. Patients with OAG are more sensitive to AL changes and experience more axial fluctuations during the first three months after trabeculectomy compared to patients with angle closure glaucoma [6, 28]. Some authors suggest that AL reduction can be predicted after three months by formula: AL reduction (mm) = -199 + 0.006 × IOP reduction + 0.008 × final IOP [23, 29]. Our scatter plot illustrates simultaneous change of AL and IOP and showed significant correlation in IOP and AL decrease through time ($r = 0.255$; $p = 0.049$; $p < 0.05$) with implication to visual acuity.

Trabeculectomy, non-penetrating surgery and drainage devices usually cause AL, ACD or keratometry changes that last more than one year, but significant enough to affect visual acuity, IOL power calculation accuracy and refractive outcomes after combined or cataract surgery. After trabeculectomy, every 0.1 mm change in AL leads to an average 0.25 diopter change in IOL power in emmetropic eye. This error was to 0.18 diopter in a very long eye (30 mm) and increases to 0.38 diopter in very short eye (20 mm). Other parameter, ACD change of 0.12 mm would result in a change of 0.06 diopter in refractive error for posterior chamber IOL [5, 6, 28–31].

CONCLUSION

Trabeculectomy is the most common surgical procedure for glaucoma management, which may significantly influence ocular biometry and may last more than one year. ACD and AL change can be significant enough to affect visual acuity, the accuracy of IOL power calculation and refractive outcomes after combined or future cataract surgery. Our biometric parameters analysis showed significant postoperative difference in ACD (for 1.1 mm during time interval) and AL decrease (0.39 ± 1.27 mm on the seventh day) with spontaneous recovery two months after operation. There was a difference in the postoperative visual acuity compared to the preoperative (worsening for two lines at Snellen chart) with spontaneous recovery at the end of follow up. As our continuous effort is to develop new procedures and improve actual, to avoid problems associated with glaucoma filtering surgery, we must strive to improve our surgical outcome, modify surgical technique, and develop strategies to optimize visual acuity and accuracy of IOL power calculation in order to achieve the best results for our patients.

NOTE

These results were presented in Marija Radenković's master thesis and parts of these results were presented at the XVIII Congress of Serbian Ophthalmologists held on September 21–24, 2017 in Aranđelovac, Serbia.

Conflict of interest: None declared.

REFERENCES

1. Sarkisian S. Optimizing Techniques for Filtering Surgery to Improve Visual Outcomes. Glaucoma Today. 2011. Available from: http://glaucomatoday.com/2011/06/.
2. Lee WW. Ultrasound vs. Optical Biometry. 2011. Available from: http://www.ophthalmologyweb.com.
3. Shahzad HSF, Patel A, O'Brien C, DelMonte D. Biometry for Intra-Ocular Lens (IOL) power calculation. 2017. Available from: http://eyewiki.aao.org/.
4. Sahin A, Hamrah P. Clinically relevant biometry. Curr Opin Ophthalmol. 2012; 23(1):47–53.
5. Kara N, Baz O, Altan C, Satana B, Kurt T, Demirok A. Changes in choroidal thickness, axial length, and ocular perfusion pressure

accompanying successful glaucoma filtration surgery. Eye. 2013; 27(8):940–5.
6. Alvani A, Pakravan M, Esfandiari H, Safi S, Yaseri M, Pakravan P. Ocular Biometric Changes after Trabeculectomy. J Ophthalmic Vis. Res. 2016; 11(3):296–303.
7. Cunliffe IA, Dapling RB, West J, Longstaff S. A prospective study examining the changes in factors that affect visual acuity following trabeculectomy. Eye. 1992; 6 (Pt 6):618–22.
8. Stewart WC, Shields MB. Management of anterior chamber depth after trabeculectomy. Am J Ophthalmology. 1988; 106(1):41–4.
9. Goins K, Smith T, Kinker R, Lewis J. Axial anterior chamber depth after trabeculectomy. Ophthalmologica. 1990; 200(4):177–80.
10. Cvetković D. Epidemiologija IOP-a i glaukoma. 2008. Toco Bel: Belgrade; 2008. p. 3–4.
11. Ehrnrooth P, Lehto I, Puska P, Laatikainen L. Long-term outcome of trabeculectomy in terms of intraocular pressure. Acta Ophthalmol Scand. 2002; 80(3):267–71.
12. Edmunds B, Thompson JR, Salmon JF, Wormald RP. The National Survey of Trabeculectomy. II. Variations in operative technique and outcome. Eye. 2001; 15(Pt 4):441–4.
13. Shaarawy T, Grehn F, Sherwood M. WGA guidelines on design and reporting of glaucoma surgical trials. 1st ed. Amsterdam: Kugler publications; 2009.
14. Ringvold A. Epidemiology of exfoliation syndrome and exfoliative glaucoma.Exfoliation Syndrome and Exfoliative Glaucoma. 2nd ed. Publicomm. 2012.
15. Andrikopoulos GK, Alexopoulos DK, Gartaganis SP. Pseudoexfoliation syndrome and cardiovascular diseases. World J Cardiol. 2014; 6(8):847–54.
16. Cook C, Foster P. Epidemiology of glaucoma: what's new? Can J Ophthalmol. 2012; 47(3):223–6.
17. Claridge KG, Galbraith JK, Karmel V, Bates A. The effect of trabeculectomy on refraction, keratometry and corneal topography. Eye. 1995; 9(Pt 3):292–8.
18. Hugkulstone CE. Changes in keratometry following trabeculectomy. Br J Ophthalmol. 1991; 75(4):217–8.
19. Stewart WC, Shields B. Management of Anterior Chamber Depth After Trabeculectomy. Am J Ophthalmol. 1988; 106(1):41–4.
20. Husain R, Li W, Gazzard G, Foster PJ, Chew PT, Oen FT, et al. Longitudinal changes in anterior chamber depth and axial length in Asian subjects after trabeculectomy surgery. Br J Ophthalmol. 2013; 97(7):852–6.
21. Alvani A, Pakravan M, Esfandiari H, Yaseri M, Yazdani S, Ghahari E. Biometric Changes After Trabeculectomy with Contact and Non-contact Biometry. Optom Vis Sci. 2016; 93(2):136–40.
22. Man X, Chan NC, Baig N, Kwong YY, Leung DY, Li FC, et al. Anatomical effects of clear lens extraction by phacoemulsification versus trabeculectomy on anterior chamber drainage angle in primary angle-closure glaucoma (PACG) patients. Graefes Arch Clin Exp Ophthalmol. 2015; 253(5):773–8.
23. Francis BA, Wang M, Lei H, Du LT, Minckler DS, Green RL, et al. Changes in axial length following trabeculectomy and glaucoma drainage device surgery. Br J Ophthalmol. 2005; 89(1):17–20.
24. Nemeth J, Horoczi Z. Changes in the ocular dimensions after trabeculectomy. Int Ophthalmol. 1992; 16(4–5):355–7.
25. Cashwell LF, Martin CA. Axial length decrease accompanying successful glaucoma filtration surgery. Ophthalmology. 1999; 106(12):2307–11.
26. Pakravan M, Alvani A, Yazdani S, Esfandiari H, Yaseri M. Intraocular lens power changes after mitomycin trabeculectomy. Eur J Ophthalmol. 2015; 25(6):478–82.
27. Kook MS, Kim HB, Lee SU. Short-term effect of mitomycin-C augmented trabeculectomy on axial length and corneal astigmatism. J Cataract Refract Surg. 2001; 27(4):518–23.
28. Pakravan M, Alvani A, Esfandiari H, Ghahari E, Yaseri M. Post-trabeculectomy ocular biometric changes. Clin Exp Optom. 2017; 100(2):128–32.
29. Saeedi O, Pillar A, Jefferys J, Arora K, Friedman D, Quigley H. Change in choroidal thickness and axial length with change in intraocular pressure after trabeculectomy. Br J Ophthalmol. 2014; 98(7):976–9.
30. Tan HY, Wu SC. Refractive error with optimum intraocular lens power calculation after glaucoma filtering surgery. J Cataract Refract Surg. 2004; 30(12):2595–7.
31. Üretmen Ö, Ateş H, Andaç K, Deli B. Axial Length Changes Accompanying Successful Nonpenetrating Glaucoma Filtration Surgery. Ophthalmologica. 2003; 217(3):199–203.

Биометријске промене ока после трабекулектомије

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САЖЕТАК

Увод/Циљ Трабекулектомија је конвенционална филтрациона процедура у хируршком лечењу глаукома. Чак и после успешно изведене трабекулектомије пацијентима може бити редукована видна оштрина. Студије из 1991. показују да промене видне оштрине настају услед промене закривљености рожњаче и дубине предње коморе. Промена дубине предње коморе за 1 *mm* резултује променом за око две диоптрије у рефрактивној сфери. Истовремено са променама на предњем сегменту, ефекат антиглаукомних операција се може манифестовати и на задњем сегменту ока: дебљини хороидеје, дужини ока и окуларној перфузији. Биометријом се потврђује смањење дужине ока после трабекулектомије, израженије код преоперативно виших вредности очног притиска и у првој постоперативној недељи, са спонтаним враћањем на преоперативне вредности годину дана после интервенције. Студија је спроведена на Клиници за очне болести у Нишу на 60 оперисаних пацијената са глаукомом отвореног угла, ради утврђивања разлике пре/постоперативних вредности биометрије.

Методе У овом раду коришћена је ретроспективно-проспективна биометријска анализа пацијената оперисаних од глаукома.

Резултати Дубина предње очне коморе се значајно разликује током посматраног периода праћења – за 1,1 *mm* у првој недељи ($p < 0,0001$) у целокупној групи и по типовима глаукома. Просечна вредност дужине ока се значајно разликује током посматраног периода праћења – краћа је за 0,39 *mm* у првој постоперативној недељи ($p < 0,05$).

Закључак Анализом биометријских параметара утврђена је постоперативна разлика у вредностима биометрије са спонтаним опоравком. Постоји разлика постоперативне видне оштрине пацијената у односу на преоперативну са спонтаним опоравком на крају праћења.

Кључне речи: глауком; трабекулектомија; биометрија; дубина предње коморе; дужина ока

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Craniofacial measures and minor physical anomalies in patients with schizophrenia in a cohort of Serbian population

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SUMMARY

Introduction/Objective Craniofacial dysmorphology has been shown as the most prominent among physical anomalies in schizophrenia patients. The aim of the present study was to investigate the frequency of craniofacial anomalies in Serbian schizophrenia patients.

Methods A list of 27 minor physical anomalies (modified Waldrop scale) and nine ratios of craniofacial measures was used to detect the presence of craniofacial dysmorphology in 126 schizophrenia patients and 124 healthy controls.

Results Compared to the healthy subjects, schizophrenia patients had significantly higher rates of the following minor physical anomalies: fine hair, two or more hair whorls, fused eyebrows, wide nose basis, low-seated ears, high steepled and high flat palate, and furrowed tongue (most prevalent were vertical fissures and diffusely distributed fissures) with significance of $p \leq 0.001$. The best predicting parameters for distinguishing between schizophrenics and controls were the inner canthus distance, the outer canthus distance, hair whorls (all at level $p = 0.000$), and high steepled palate ($p \leq 0.001$).

Conclusion The results of the present study confirm the neurodevelopmental concept of schizophrenia, being potentially useful for further psychiatric-anthropological research. Clinical significance is reflected in the possibility of monitoring the potential mental illness in childhood through potential ectodermal markers, as well as the possibility of their comparison with the psychological profile in early adolescence.

Keywords: minor malformations; phenogenetic variants; facial morphometry; schizophrenia phenotype; facial disproportion

INTRODUCTION

In the era of technologically advanced and highly precise diagnostic methods, schizophrenia remains a disease whose etiology and pathophysiology remain poorly understood. There are several theories about schizophrenia origin, among which the neurodevelopmental theory is the widely accepted one [1, 2, 3]. During the first trimester of fetal development, the ectoderm and its derivatives develop intensively – the epidermis, hair, nails, sweat glands, tooth enamel, brain, lining of the mouth, anus and nostrils. Due to the common embryonic origin of the face and the brain, minor physical anomalies (MPAs) could be considered as potential indices of brain abnormalities linked to schizophrenia [2, 3]. MPAs were defined for the first time by Marden [4] as anomalies which are neither of medical nor cosmetic consequence to the patient, occurring in diverse body regions, such as craniofacial region, mouth, eye, ear, hand and feet region. MPAs in behavioral disorders were initially studied in children as associated anomalies with behavioral disturbances; later, they were recognized as indicative in schizophrenia disorders [1, 5–8].

MPAs are thought to have prenatal origin, being the consequences of ectodermal malformation, either caused by different noxae, or genetically conditioned. To date, many authors reported an excess of MPAs' occurrence in people with autism, attention deficit hyperactivity disorder, epilepsy, fetal alcoholic syndrome, schizophrenia, and even pedophilia [9–15]. MPAs were found to be positively associated with reduced prefrontal volume and enlarged basal ganglia volumes [16]. According to the vascular-inflammatory theory of schizophrenia origin, genetically modulated inflammatory response damages the microvascular system of the brain in reaction to environmental agents (infections, hypoxia and physical trauma), leading to the abnormalities of the central nervous system's metabolism [17]. However, this theory cannot explain the higher prevalence of MPAs in schizophrenia patients, nor in their first-degree relatives [18].

The use of the Waldrop scale brought along some issues, among them the absence of distinction between minor malformations, which arise during organogenesis, and phenogenetic variants, which appear after organogenesis. Some authors emphasized the

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need to make this distinction in order to indicate the time and nature of brain adverse events [10, 19]. The minor malformations are qualitative defects of embryogenesis being always abnormal, while the phenogenetic variants are quantitative defects of final morphogenesis. Organogenesis involves thresholds, i.e. all-or-none traits, while phenogenesis (the final morphogenesis) represents the process of developmental "fine-tuning" [19].

Several studies indicated that among patients with schizophrenia, the craniofacial region had the MPAs more commonly than the other regions [2, 3, 6]. Gourion et al. [6] found the facial asymmetry, cleft palate and multiple hair whorls as the most discriminating among other MPAs. In the study by Lane et al. [3], the most prevalent MPAs were the high palate, palate ridge, supraorbital ridge and epicanthus. Trixler et al. [20] found that specific anomalies of the mouth and head, such as furrowed tongue, flat occiput, and primitive shape of the ears, might have more relevance to the hypothetical neurodevelopmental failure than the cumulative prevalence of MPAs did.

The objective of this study is to determine the prevalence of craniofacial MPAs in schizophrenia patients in this region of Europe. We sought to compare the occurrence of MPAs between schizophrenia patients and healthy controls, and to determine predictors of schizophrenia among MPAs.

METHODS

Subjects

The study group consisted of 126 schizophrenic inpatients (68 males and 58 females, mean age 35.02 ± 10.31 years) who had been hospitalized at the Clinical Center of Vojvodina in Novi Sad, Department of Psychiatry, in the period January 2012 – December 2015. All the patients satisfied the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for a diagnosis of schizophrenia (APA 2013) on the basis of case records review (Diagnostic and Statistical Manual of Mental Disorders, DSM-5, 2013). Potential subjects were excluded if they had a history of drug or alcohol abuse, a neurological disorder (seizure disorder, head trauma, multiple sclerosis, etc.), or any signs of mental retardation or somatic disorder with neurological components. The mean age of the first neuroleptic treatment was 30.23 years (SD = 8.91, range = 16–53), the mean length of illness since the first psychotic episode was 11.77 years (SD = 9.87, range = 0–36), and the mean age at time of study was 42.70 years (SD = 12.70, range = 19–64). Family history of mental disorders was present in 43 (33.96%) patients, and 24 (18.86%) of them had at least one suicide attempt. The group of patients with schizophrenia included 75 subjects with undifferentiated sub-type, 24 with paranoid sub-type, 24 with schizoaffective disorder, and three with disorganized sub-type.

The control group comprised 124 healthy subjects (61 males and 63 females, the mean age being 32 ± 13.38 years). All the subjects were interviewed by a trained psychiatrist using Structured Clinical Interview for DSM-5 Non-patient Version to ensure the absence of major mental disorders.

All the subjects were of Caucasian race. Migrants from other regions or states were excluded to avoid a geographical bias. Written informed consent was obtained from all the participants. The study was approved by the Ethics Committee of the Clinical Center of Vojvodina and is in compliance with the Helsinki Declaration.

Assessment of minor physical anomalies

The presence of MPAs in the craniofacial region was assessed using a modified Waldrop scale, incorporating 13 elements from the original Waldrop scale, elements listed in other author's scale and new elements that we added in this modified scale (Tables 1 and 2) [5]. The assessment of MPAs was done qualitatively (present or absent) without scores being used. Craniofacial measures were obtained using a sliding caliper, a spreading caliper, and nonstretchable measuring tape (Holtain Ltd., Croswell, UK) to the nearest 0.1 cm, as distances between standard anthropological landmarks. Body height was measured using GPM anthropometer to the nearest 0.1 cm (Sieber&Hegner, Zürich, Switzerland) with the subject standing straight up without shoes. Head circumference was measured using a flexible but non-stretchable tape (Holtain Ltd.), to the nearest 0.1 cm; it was measured from just above the glabella to the most posterior prominent point of the occipital bone.

The inner canthus distance (ICD) and the outer canthus distance (OCD) were put into the ratio because of the statistical reasons.

In order to determine which type of tongue fissures was the most prominent one, several items were added to the modified Waldrop scale: continuous longitudinal fissure, discontinuous longitudinal fissure, one central and

Table 1. Student's t-test, comparison of continuous variables between patients with schizophrenia and controls

Variables	Patients (n = 123)	Control (n = 108)	p	95% CI
	X ± SD (cm)			
Head circumference[1]	54.98 ± 2.8	56 ± 2.22	0.002*	0.769–0.944
Inner canthus distance[1]	3.38 ± 0.49	2.79 ± 0.45	0.000**	6.191–23.067
Outer canthus distance[2]	9.71 ± 1.24	8.78 ± 1.46	0.000**	1.292–1.885
Body height	170.55 ± 10.76	176.38 ± 10.29	0.000**	3.209–8.455

CI – confidence interval;
[1]Waldrop et al. [5];
[2]Huang et al. [8];
*p ≤ 0.0045(Bonferroni correction);
**p ≤ 0.001

Table 2. Comparison of categorical variables between patients with schizophrenia and controls, the results of the Pearson's χ^2 test

Morphological features		Patients (n = 126)	Control group (n = 124)	p	OR	95% CI
		n (%)				
Hair	Fine hair – going up soon after combing[1]	68 (54%)	41 (33.1%)	0.001**	2.373	1.422–3.963
	Fine hair – not going down after combing[1]	64 (50.8%)	23 (18.5%)	0.000**	4.533	2.559–8.03
	Two or more hair whorls[1]	82 (65.1%)	15 (12.1%)	0.000**	13.542	7.054–26.001
Eyes	Epicanthus[1]	7 (5.6%)	2 (1.6%)	0.116	3.588	0.731–17.624
	Eyebrows fused[2]	41 (32.5%)	17 (13.7%)	0.001**	3.036	1.612–5.718
	Heterochromia[2]	4 (3.2%)	1 (0.8%)	0.215	4.033	0.444–36.599
Nose	Wide nose basis[3]	65 (51.6%)	21 (16.9%)	0.000**	5.226	2.911–9.382
	Nostrils anteverted[2]	30 (23.8%)	31 (25%)	0.827	0.938	0.526–1.67
Ear	Low-seated ears – the lowest point of the earlobe in line with mouth or lower[4]	74 (58.7%)	20 (16.1%)	0.000**	7.400	4.079–13.425
	Low-seated ears - the lowest point of the earlobe in line with the area between nose and mouth[4]	48 (38.1%)	67 (54%)	0.012	0.524	0.316–0.867
	Adherent earlobes[1]	67 (53.2%)	59 (47.6%)	0.377	1.251	0.761–2.056
	Lower part of earlobes towards back	7 (5.6%)	2 (1.6%)	0.116	3.588	0.731–17.624
	Malformed ears[1]	5 (4%)	0	0.999	-	-
	Asymmetrical ears[1]	6 (4.8%)	4 (3.2%)	0.538	1.500	0.413–5.45
	Soft and pliable ears[1]	64 (50.8%)	55 (44.4%)	0.308	1.295	0.787–2.13
	Preauricular skin tag[3]	0 (0%)	3 (2.4%)	0.999	-	-
Palate	High-steepled palate[1]	72 (57.1%)	31 (25%)	0.000**	4.000	2.335–6.852
	High flat palate[1]	38 (30.2%)	21 (16.9%)	0.015	2.118	1.158–3.875
Tongue	Continuous longitudinal fissure[4]	38 (30.2%)	40 (32.3%)	0.752	0.917	0.537–1.567
	Discontinuous longitudinal fissure[4]	41 (32.5%)	40 (32.3%)	0.927	1.025	0.603–1.742
	One central and two shorter longitudinal fissures[4]	12 (9.5%)	11 (8.9%)	0.843	1.091	0.462–2.575
	Only transverse fissures[4]	9 (7.1%)	3 (2.4%)	0.093	3.129	0.827–11.847
	Transverse fissures in the last third of the tongue with a longitudinal fissure apically[4]	10 (7.9%)	5 (4%)	0.196	2.070	0.686–6.24
	Vertical fissure running along the midline and a few fissures diffusely distributed across the dorsal tongue surface[4]	20 (15.9%)	3 (2.4%)	0.001**	7.683	2.220–26.583
	Fissures diffusely distributed across the dorsal tongue surface[4]	18 (14.3%)	4 (3.2%)	0.004	5.047	1.656–15.38
	Without any fissures[1]	12 (9.5%)	14 (11.3%)	0.663	0.834	0.370–1.884
	With rough spots[1]	44 (34.9%)	39 (31.5%)	0.531	1.184	0.698–2.007

OR – odds ratio;
CI – confidence interval;
[1]Waldrop et al. [5];
[2]Ismail et al. [33];
[3]Gourion et al. [6];
[4]new/modified items;
*p ≤ 0.0018 (Bonferroni correction);
**p ≤ 0.001

two shorter longitudinal fissures, only transverse fissures, transverse fissures in the posterior third of the tongue with a longitudinal fissure apically, vertical fissure running along the midline and a few fissures diffusely distributed across the dorsal tongue surface, fissures diffusely distributed across the dorsal tongue surface, without any fissures, with rough spots.

Low-seated ears were determined using a slightly modified definition – the lowest point of the earlobe positioned in line between the nose and the mouth, or in line with the mouth or lower.

Assessment reliability

Before the statistical analysis, the interrater reliability was tested and the kappa coefficient was > 0.75 for categorical measures, and the intraclass correlation coefficient for the continuous variables was 0.5–0.9 (moderate/good reliability).

Statistical analysis

Two-tailed Fisher's exact probability or Pearson's χ^2 tests for categorical variables and Student's t-test or Mann–Whitney U test for continuous variables were used to compare variables between the two groups. We used the binary logistic regression to determine the best predictors of schizophrenia among the MPAs.

The multiple univariate logistic regression was used to determine schizophrenia predictors among MPAs. Also, all the variables with odds ratio of 1.5 or higher with $p \leq 0.05$ were put in a model for diagnosing schizophrenia based on MPAs. The model was tested using the Hosmer–Lemeshow goodness of fit statistics. Post-hoc Bonferroni corrections were done for all statistical tests used in this study to avoid error due to multiple comparisons. The IBM SPSS Statistics for Windows, version 19.0 was used for all analyses (IBM Corp., Armonk, NY, USA). We used a two-way MANOVA

Table 3. Ratios between body height and head circumference, Student's t-test

Variables	Patients (n = 123)	Control (n = 108)	p	95% CI
	X ± SD			
BH/HC	3.10 ± 0.16	3.15 ± 0.18	0.054	0.0005–0.08
BH/HC – males	3.17 ± 0.14	3.23 ± 0.15	0.018*	0.011–0.116
BH/HC – females	3.05 ± 0.15	3.06 ± 0.02	0.722	0.045 ± 0.065

HC – head circumference, BH – body height; CI – confidence interval;
*p ≤ 0.05

Table 4. Two-way MANOVA (Sex X Group) of selected continuous variables

Variable	Sex	Group	Mean	SD	p	η_p^2
Body height	female**	Research	163.37	6.12	0.000**	0.532
		Control	169.07	6.94		
	male**	Research	179.64	7.14		
		Control	183.59	7.94		
Head circumference	female**	Research	53.60	2.31	0.000**	0.178
		Control	55.33	1.92		
	male	Research	56.60	2.46		
		Control	56.68	2.32		
(Inner canthus distance + outer canthus distance)/2	female**	Research	6.61	0.84	0.002*	0.044
		Control	5.47	0.71		
	male	Research	6.71	0.80		
		Control	6.10	1.00		

SD – standard deviation; η_p^2 – partial eta-squared;
*p ≤ 0.007 (Bonferroni correction);
**p ≤ 0.001

(group × sex) to compare some of the measured continuous variables in participants within the research and control groups.

RESULTS

In the present study, four continuous variables were measured (Table 1) and 27 categorical variables were assessed (Table 2), and divided into the hair, eyes, nose, ear, palate, and tongue items. Schizophrenia patients had significantly lesser head circumference and body height, and higher values of subnasion–stomion distance, ICD, and OCD. Significantly higher rate of the following MPAs was found in schizophrenia patients: fine hair, two or more hair whorls, fused eyebrows, wide nose basis, low-seated ears (the lowest point of the earlobe in line with mouth or lower), high-steepled palate and furrowed tongue – vertical fissure running along the midline.

The head circumference put into the ratio with body height has shown a significant difference only in male patients, while in female patients and in total sample there was no statistical significance (Table 3).

A two-way MANOVA (group × sex) was used to compare the measured variables in participants between the research and control groups (Table 4). The dependent variables were reduced to three variables, due to high correlation among the continuous variables. The MANOVA yielded a significant group effect ($\Lambda = 0.648$, $F(1,126) = 14.567$, $p < 0.0001$), sex effect ($\Lambda = 0.403$, $F(1,126) = 39.767$, $p < 0.0001$) and interaction effect ($\Lambda = 0.926$, $F(1,126) = 2.150$, $p < 0.05$).

Logistic regression analysis

The entire model of multiple univariate logistic regression including all 12 predictors was statistically significant, $\chi^2 = 170.67$, $p \leq 0.001$ ($df = 13$, $n = 250$) (Table 5). Values of tolerance in collinearity diagnostics showed no significant multicollinearity between investigated predictors. This model described between 52.4% (R^2 of Cox and Snell) and 69.9% (R^2 of Nagelkerke) of variance in schizophrenia status. The Hosmer–Lemeshow test as an index of model fitness indicated a good predictive ability of this model ($p = 0.791$). As it was presented in Table 3, four of 13 investigated items made significant ($p \leq 0.0038$) independent contribution to the prediction of patient–control group status. According to this logistic regression model, significant predictors of schizophrenia were ICD, OCD, two or more hair whorls, and high-steepled palate. This model correctly classified 85.2% of patients and 91% of the comparison subjects, with the overall classification of 88.3%.

DISCUSSION

To the best of our knowledge, this is the study with the highest number of subjects in a cohort of Serbian population on the prevalence of MPAs in schizophrenic patients. The four following MPAs were indicative as predictors of schizophrenia and confirmed by the logistic regression model: ICD, OCD, two or more hair whorls, and high-steepled palate. The mouth region has been shown as highly susceptible to MPAs in schizophrenic patients, with the highest prevalence of the palate and tongue anomalies [3,

Table 5. Multiple univariate logistic regression model for the prediction of group status of schizophrenic patients and normal comparison subjects based on minor physical anomalies

Variable	Beta estimate	SE	χ^2 (df = 13)	p
Inner canthus distance	3.906	0.773	25.558	0.000**
Outer canthus distance	-1.156	0.32	13.05	0.000**
Fine hair – not going down after combing	0.853	0.501	2.894	0.089
Fine hair – going up soon after combing	0.098	0.464	0.044	0.833
Two or more hair whorls	2.049	0.521	15.475	0.000**
Eyebrows fused	0.318	0.552	0.332	0.565
Wide nose basis	0.545	0.519	1.104	0.293
Low-seated ears – the lowest point of the earlobe in line with mouth or lower	1.257	0.47	7.162	0.007
High-steepled palate	1.698	0.532	10.191	0.001**
High flat palate	1.225	0.593	4.268	0.039
Vertical fissure running along the midline and few fissures diffusely distributed across the dorsal tongue surface	1.425	1.001	2.028	0.154
Fissures diffusely distributed across the dorsal tongue surface	1.847	0.983	3.529	0.060
Constant	-4.882	1.625		

SE – standard error; df – degree of freedom;
*p ≤ 0.0038 (Bonferroni correction);
**p ≤ 0.001

6, 7, 21]. Our results are consistent with findings confirming significantly higher rates of the high-steepled palate among schizophrenic patients (57.1% *vs.* 25%). Facial-cerebral morphogenesis has been postulated as a primarily midline process, and dysmorphology in schizophrenia patients' face affects principally the midfacial region, including the development of the palate [2]. The midfacial region is populated with the cells of the cranial neural crest during the embryogenesis, which could explain the coincidence of midfacial minor anomalies and schizophrenic disorder. The maxilla and mandible are derived from the cranial neural crest cells from the diencephalon and anterior part of the mesencephalon, while the nasal processes are derived from the diencephalon and anterior part of the mesencephalon [22]. Gourion et al. [6] reported that the latter could be brought into relation with the wider nasal base [23].

Six minor malformations were significantly more common in the group of schizophrenia patients than in the control group: fine hair, two or more hair whorls, high palate, furrowed tongue eyebrows fused, wide nose basis ($p < 0.01$). Most studies showed furrowed tongue to be a significant marker of schizophrenia without respect to the type of furrows; Scutt et al. [24] reported higher rate of large tongue, while other studies scored randomly furrowed tongue, transversely furrowed tongue or tongue with smooth-rough spots [25]. Our study revealed significantly higher prevalence of only one tongue features type in schizophrenic patients: vertical fissure running along the midline and few fissures diffusely distributed cross the dorsal tongue surface (15.9 *vs.* 2.4%).

Some epidemiological studies suggest that height and schizophrenia are inversely correlated [26, 27]. Our study showed that both men and women were significantly shorter in the research group, with large effect size. Bacanu et al. [28] explained that the height and schizophrenia disorder are likely to have mostly overlapping genetic causes of discordant effect. Body height was used to relativize the value of head circumference, and this ratio was significantly higher in a study by Mishra et al. [29], which is also in line with results of Huang et al. [8]. Our study confirmed these findings only in male population – circumference relative to body height has been revealed as a significantly greater ratio in male schizophrenia patients, but without any statistically significant difference in the total sample.

Association between brain anomalies and hair variations could be justified by their common developmental origin. Our results showed that 65.1% of schizophrenic patients have two or more hair whorls, which is in consonance with the results of Gourion et al. [6]. Fine electric hair was observed more often in the schizophrenic patients regardless of the sex, but it showed weak predictive power. Gourion et al. [6] also showed significantly higher rate of fine electrical hair in schizophrenic patients.

There were no significant differences between the groups regarding the eye features except of the fused eyebrows, which were found in more than 30% of schizophrenic patients. Gourion et al. [6] reported fused eyebrows as a statistically insignificant item. Concerning the measurements in the eye region, Elizarrarás-Rivas et al. [30] found significantly greater ICD and OCD in schizophrenia patients in the Mexican population, as did we in our population – ICD and OCD were statistically greater. The position of the ears was defined differently in our study than in most of the previous studies, considering the level of the lowest point of the earlobe instead [6, 31]. The majority of schizophrenic patients (58.7%) had the lowest point of the earlobe in line with mouth or lower, with high statistical significance ($p = 0.000$). The statistically significant presence of low-seated ears in schizophrenia patients was previously found by Gourion et al. [6]. However, this item was recognized as insignificant in the studies of the Chinese and the Bulgarian population [20, 31]. Other ear items in our study did not differ significantly between the groups.

A study on MPAs by Ivković et al. [32] in a cohort of the Serbian population included less subjects and studied just the MPAs of the mouth and palate. They showed that there

was no statistically significant difference between schizophrenia patients and control subjects in observed MPAs.

Regarding the time and nature of abnormalities, our study indicates that they occur during and after organogenesis, since minor malformations and phenogenetic variants both differ between schizophrenia patients and control subjects. Noted higher frequency of minor malformations and/or phenogenetic variants in the research group cannot determine with certainty the exact time of brain adverse event in the prenatal period, but could point out the potential prenatal period of higher neurological sensitivity.

CONCLUSION

The results of this study contribute to the existing body of knowledge and support the neurodevelopmental hypothesis of schizophrenia. The inner canthus distances, outer canthus distances, two or more hair whorls, and high-steepled palate showed the best discriminative power.

Conflict of interest: None declared.

REFERENCES

1. Compton MT, Walker EF. Physical manifestations of neurodevelopmental disruption: are minor physical anomalies part of the syndrome of schizophrenia? Schizophr Bull. 2009; 35(2):425–36.
2. Waddington JL, Lane A, Scully P, Meagher D, Quinn J, Larkin C, et al. Early cerebro-craniofacial dysmorphogenesis in schizophrenia: a lifetime trajectory model from neurodevelopmental basis to 'neuroprogressive' process. J Psychiatr Res. 1999; 33(6):477–89.
3. Lane A, Kinsella A, Murphy P, Byrne M, Keenan J, Colgan K, et al. The anthropometric assessment of dysmorphic features in schizophrenia as an index of its developmental origins. Psychol Med. 1997; 27(5):1155–64.
4. Marden PM. Congenital anomalies in the newborn infant, including minor variations. J Pediatr. 1964; 64(3):357–71.
5. Waldrop MF, Pedersen FA, Bell RQ. Minor physical anomalies and behavior in preschool children. Child Dev. 1968; 39(2):391–400.
6. Gourion D, Goldberger C, Bourdel MC, Bayle FJ, Loo H, Krebs MO. Minor physical anomalies in patients with schizophrenia and their parents: prevalence and pattern of craniofacial abnormalities. Psychiatry Res. 2004; 125(1):21–8.
7. Green MF, Satz P, Gaier DJ, Ganzell S, Kharabi F. Minor physical anomalies in schizophrenia. Schizophr Bull. 1989; 15(1):91–9.
8. Huang CJ, Chiu HJ, Tsuo-Hung L, Wang HF, Kuo SW, Chen SF, et al. Significance of morphological features in schizophrenia of a Chinese population. J Psychiatr Res. 2010; 44(2):63–8.
9. Manouilenko I, Eriksson JM, Humble MB, Bejerot S. Minor physical anomalies in adults with autism spectrum disorder and healthy controls. Autism Res Treat. 2014; 2014:743482.
10. Trixler M, Tenyi T, Csabi G, Szabo G, Mehes K. Informative morphogenetic variants in patients with schizophrenia and alcohol-dependent patients: beyond the Waldrop scale. Am J Psychiatry. 1997; 154(5):691–3.
11. Dyshniku F, Murray ME, Fazio RL, Lykins AD, Cantor JM. Minor physical anomalies as a window into the prenatal origins of pedophilia. Arch Sex Behav. 2015; 44(8):2151–9.
12. Csabi G, Zsuppan R, Jeges S, Tenyi T. Minor physical anomalies are more common with idiopathic epilepsy. Neuropsuchopharmacol Hung. 2014; 16(3):115–20.
13. Fountoulakis KN, Panagiotidis P, Gonda X, Kimiskidis V, Nimatoudis I. Neurological soft signs significantly differentiate schizophrenia patients from healthy controls. Acta Neuropsychiatr. 2018; 30(2):97–105.
14. Fazio RL. Toward a Neurodevelopmental Understanding of Pedophilia. 2018; 15(9):1205–7.
15. Myers L, Anderlid BM, Nordgren A, Willfors C, Kuja-Halkola R, Tammimies K, et al. Minor physical anomalies in neurodevelopmental disorders: a twin study. Child Adolesc Psychiatry Ment Health. 2017; 11:57.
16. Dean K, Fearon P, Morgan K, Hutchinson G, Orr K, Chitnis X, et al. Grey matter correlates of minor physical anomalies in the AeSOP first-episode psychosis study. Br J Psychiatry. 2006; 189:221–8.
17. Hanson DR, Gottesman II. Theories of schizophrenia: a genetic-inflammatory-vascular synthesis. BMC Med Genet. 2005; 6:7.
18. Hajnal A, Csabi G, Herold R, Jeges S, Halmai T, Trixler D, et al. Minor physical anomalies are more common among the first-degree unaffected relatives of schizophrenia patients – Results with the Méhes Scale. Psychiatry Res. 2016; 237:224–8.
19. Opitz JM. Invited editorial comment: Study of minor anomalies in childhood malignancy. Eur J Pediatr. 1985; 144(3):252–4.
20. Trixler M, Tenyi T, Csabi G, Szabo R. Minor physical anomalies in schizophrenia and bipolar affective disorder. Schizophr Res. 2001; 52(3):195–201.
21. Delice M, Gurbuz O, Oflezer C, Kurt E, Mandali G. Palate size and shape in schizophrenia. Psychiatry Res. 2016; 244:273–8.
22. Serbedzija GN, Bronner-Fraser M, Fraser SE. Vital dye analysis of cranial neural crest cell migration in the mouse embryo. Development. 1992; 116(2):297–307.
23. Lin Y, Ma X, Deng W, Han Y, Li M, Liu X, et al. Minor physical anomalies in patients with schizophrenia in a Chinese population. Psychiatry Res. 2012; 200(2–3):223–7.
24. Scutt LE, Chow EWC, Weksberg R, Honer WG, Basset AS. Patterns of dysmorphic features in schizophrenia. Am J Med Genet. 2001; 105(8):713–23.
25. Akabaliev VH, Sivkov ST. Minor physical anomalies in schizophrenia. Folia Med (Plovdiv). 1998;40(2):39–45
26. Gunnell D, Harrison G, Whitley E, Lewis G, Tynelius P, Rasmussen F. The association of fetal and childhood growth with risk of schizophrenia. Cohort study of 720,000 Swedish men and women. Schizophr Res. 2005; 79(2–3):315–22.
27. Zammit S, Rasmussen F, Farahmand B, Gunnell D, Lewis G, Tynelius P, et al. Height and body mass index in young adulthood and risk of schizophrenia: a longitudinal study of 1 347 520 Swedish men. Acta Psychiatr Scand. 2007; 116(5):378–85.
28. Bacanu SA, Chen X, Kendler KS. The genetic overlap between schizophrenia and height. Schizophr Res. 2013; 151(1–3):226–8.
29. Mishra V, Sharma S, Kulsreshtha A, Kumar V, Guruna KC. Anthropometerical Association of the Craniofacial Dysmorphology with Schizophrenia. J Clin Diagn Res. 2012; 6(10):1620–3.
30. Elizarrarás-Rivas J, Fragoso-Fererra R, Cerdan-Sanchez LF, Ramos-Zepeda R, Barajas-Barajas LO, Troyo-Sanroman R, et al. Minor physical anomalies and anthropometric measures in schizophrenia: a pilot study from Mexico [Letter to the editor]. Schizoph Res. 2003; 62(3):285–7.
31. Akabaliev VH, Sivkov ST. Sexual dimorphism in minor physical anomalies in schizophrenic patients and normal controls. Compr Psychiatry. 2003; 44(4):341–8.
32. Ivkovic M, Damjanovic A, Paunovic VR. Neurodevelopmental correlates in schizophrenia. Srp Arh Celok Lek. 2003; 131(7–8):294–9.
33. Ismail B, Cantor-Graae E, McNeil TF. Minor physical anomalies in schizophrenic patients and their siblings. Am J Psychiatry. 1998; 155(12):1695–702.

Краниофацијалне мере и „минор“ физичке аномалије код болесника са схизофренијом у кохорти српске популације

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САЖЕТАК

Увод/Циљ Дисморфологија краниофацијалних структура показала се као најистакнутија међу физичким аномалијама код схизофрених болесника. Циљ наше студије је да истражимо учесталост краниофацијалних аномалија код схизофрених болесника у Србији.

Метод Испитивану групу чинило је 126 схизофрених болесника, а контролну 124 здрава испитаника. Анализирано је 27 „минор“ физичких аномалија (модификованих по Валдроповој скали) и девет вредности међусобних односа мерених варијабли.

Резултати У односу на здраве испитанике схизофрени болесници су имали значајно већу стопу следећих „минор“ физичких аномалија: танка длака косе, два или више вртлога у власишту косе, спојене обрве, широка база носа, ниско постављене ушне шкољке, високо уско непце, као и избразданост језика (најучесталије су биле уздужне и дифузно постављене бразде) са статистичком значајношћу од $p \leq 0{,}001$. Статистички посматрано, најбољи предиктивни значај за разликовање схизофрене и контролне групе имале су следеће варијабле: раздаљина између унутрашњих углова очију, раздаљина између спољашњих углова очију, вртлози у власишту косе (сва три параметра на нивоу значајности од $p = 0{,}000$) и високо непце ($p \leq 0{,}001$).

Закључак Резултати наше студије подржавају неуроразвојни концепт настанка схизофреније, уз могућност примене у даљим психијатријско-антрополошким истраживањима. Клинички значај се огледа у могућности праћења могућих ектодермалних маркера душевних болести у детињству, те њиховог поређења са психолошким профилом у раној адолесценцији.

Кључне речи: „минор“ малформације; феногенетске варијанте; морфометрија лица, схизофрени фенотип; диспропорција лица

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Creating social marketing strategy on the internet within preventive health care – human papilloma virus vaccination campaign

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SUMMARY

Introduction/Objective The main aim of the paper is to develop a foundation for creating internet social marketing strategy in preventive health care, through research and campaign for vaccination against human papillomavirus (HPV).

The aim of the study was to introduce a strategic approach of social marketing on social networks, for vaccination against HPV campaign in Serbia.

Methods Quantitative research was conducted through the internet in December of 2016, using the survey method. Participants in the study were parents whose children were candidates for the vaccination.

Results The research has shown that nearly one third of respondents do not know what HPV is, and about the same number of respondents know that HPV causes cancer. In addition, only 14.5% of respondents know that HPV is the most common transmitted disease in the world. With adequate awareness of safety, 97% of respondents would decide to vaccinate their children. Only 39% of parents could afford the vaccination, although opinion change is caused by the information about the price of the HPV vaccine. Consequently, 97.5% of the respondents would opt for vaccination in the case that it is free.

Conclusion Based on the results, and compared with best practices of other countries, we provided a marketing strategy via social networks. The campaign focuses on the raising awareness of the need for HPV vaccination and cancer prevention, including disseminating information to the target population, through social networks.

Keywords: human papillomavirus; vaccination; prevention; social marketing; strategic marketing on the internet

INTRODUCTION

Practical experience and evidence of health promotion programs, campaigns, and national strategies for key disease groups, such as HPV virus infection, have increased throughout Europe. Enhancing social behavioral research could provide a larger basis of evidence, as the foundation for actions in prevention. Start-up guidance through programs and campaigns for the prevention of human papillomavirus (HPV), through vaccination, is especially important for young people.

There are two basic groups gathered around the interest – the fight against HVP. The first group is teenagers, during the period of maturity for vaccination, and the second group is the parents of those teenagers, who have the need and responsibility to protect their children. For young people, programs for the prevention of HVP infection can include peer education, inclusion of youth organizations, and school health literacy programs. However, more significant effects could be achieved through social networks.

HPV infection is the most important risk factor and a necessary condition for the development of cervical cancer [1]. In Serbia, an average of 482 women die of cervical cancer each year, based on the estimates of the International Agency for Research on Cancer and the European Network of Registries for Cancer, Serbia remains in the group of European countries with the highest rates of illness and dying from cervical cancer.

HPV is also responsible for more than 90% of cases of colon cancer, 71% of cancer of the genital organs in both sexes, and 72% of cases of lung and throat cancer, according to the US National Cancer Institute. To date, more than 120 types of HPV have been identified. Some of them (about 40 types of viruses) are sexually transmitted and lead to infection of sex organs and the anogenital region of both women and men [2].

HPV is so widespread that most adults (about 70% of people) have had an HPV infection in their lifetime. Primary HPV infection usually does not exhibit any symptoms and most people create antibodies without being aware that they

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have been infected. In some cases, HPV infection can be maintained without any symptoms for many years. It is therefore very difficult to say with certainty when and how the infection occurred.

A study of 194 studies involving over one million women with normal cytology showed that the global prevalence of HPV infection in the world was 11.7% [3]. At younger ages, it is very high and reaches the peak at 20–25 years. In most women infected with HPV, the immune system will generate antibodies and overcome viral infection within 6–24 months to create antibodies and to overcome viral infection. Spontaneous disappearance of the infection occurs in about 85–90% of cases, which is confirmed by the low prevalence of latent HPV infection in women over 30 years of age [4]. In a small number of women, the infection persists (10–15%), and this occurs mainly when it is caused by high-risk HPV types [5].

There are several ways to prevent the HPV infection. This includes vaccination against HPV, change in sexual behavior, smoking prevention, and other health education activities. In this study, the focus of research relates to the vaccination against HPV. In Serbia, the 2020 Health Policy supports sustained efforts to combat infectious diseases such as HPV. So far, there have been no vaccine advocacy campaigns against HPV in Serbia, but have been implemented for many years in developed countries of the world.

The use of HPV vaccines prevents the emergence of persistent infection, precancerous changes, and malignancies caused by certain types of HPV. Based on data on the high incidence of HPV infection and the high risk of developing an infection immediately after starting sexual activity, the highest level of protection is achieved by the use of the vaccine before the onset of sexual activity. For this reason, vaccination is recommended at the age of 10–14 years. The vaccine is not recommended for girls younger than nine years, because in this group the immunogenicity and efficacy of the vaccine have not been investigated. Vaccine efficacy in women over 26 years of age has not been confirmed and additional research is ongoing. High protection is achieved by immunization in women of the age of 14 or 15 to 26 years if they have not started sexual activity [6]. From the introduction of the vaccine in Europe in 2006, the girls were at the center of attention. However, the question of the usefulness of the vaccine for boys was also raised – only the effectiveness of Gardasil vaccine was evaluated and the results showed it to be as effective as it is for girls, if not more so.

At the moment, there are two vaccines – Gardasil (Merck & Co., Kenilworth, NJ, USA) and Cervarix (GlaxoSmith-Kline plc., London, UK). According to the European Centre for Disease Prevention and Control report, both vaccines protect against high-risk types of HPV 16 and 18, which are believed to be cancer susceptors in 73% of cases in Europe. Gardasil, in addition, protects against HPV types 6 and 11, which are the most common causes of condyloma of the genital organs. Vaccines are given in three doses within six months. Both vaccines are registered in Serbia, but the Health Insurance Fund does not cover them; they are quite expensive for ordinary citizens, and not easily obtained in clinics and pharmacies.

The role of parents and health workers is crucial, as the vaccine is not mandatory, and parents' approval is required. Of the 29 European Union member states, Norway, and Iceland, vaccination against HPV is carried out in 19 countries. The rate of vaccination, however, is uneven and ranges 17–84%. A full coverage of over 80% was in Portugal and the UK in 2010.

The governments of the European Union countries, as well as the government of Serbia, currently only spend a small part of the health budget for health promotion and disease prevention – around 3% [7]. According to Serbia's current Strategy for Health Development until Year 2020, real health benefits can be achieved at affordable costs and within resource constraints if effective strategies are adopted.

The main objective of the study is to develop a foundation for the promotional strategy for increasing public awareness of the capabilities, significance, and safety of HPV prevention through immunization. The specific aims of the study were to determine the correlation between the knowledge about HPV virus and decisions of parents to vaccinate their children, as well as to determine whether the price of the vaccine is a significant factor that affects parents to vaccinate their children.

METHODS

Participants

The population for this research were parents with children up to 15 years of age. The sample comprised 200 participants, representatives of the general population of Serbia. The survey involved 172 women (86%) and 28 men (14%).

Twenty-one percent of the respondents had only one child, 72% had two children, and only 7% had three children. The total number of children is 228, of which 126 boys (55.26%) and 102 girls (44.74%).

Methods

In this research we applied quantitative research using a survey method. The instrument used for this research was the questionnaire given in Appendix A. The questionnaire contains 15 questions. The role of the first group of three questions was to collect demographic data. The next six questions were designed to examine the participants' familiarity with different aspects of HPV. The last group of six questions was intended to collect data on intentions of parents to vaccinate their children.

Data collection was performed using the online questionnaire, which was uploaded to Google Drive. The link to the questionnaire was forwarded to the parent sample, set to the active forums.

The sample in this study had characteristics similar to those in the population. In addition, it was a stratified sample, since it was derived from the target population as a subset of the baseline, according to the criteria of the posi-

Appendix A
RESEARCH QUESTIONNAIRE

1. Male or Female: M F
2. Number of children (male or female): _____
3. How old are the children: _____
4. Do you know what HPV virus is? Yes No
5. Do you know that HPV is the most common reason for diseases in the world? Yes No
6. Do you know that HPV virus has direct impact on triggering malignant diseases? Yes No
7. Do you know that HPV virus can cause cervical cancer in women, as well as cancer in men? Yes No
8. Do you know that HPV virus is the key cause for intimacy diseases in women and men? Yes No
9. Scientific research has proved that HPV vaccine is efficient in preventing diseases. In that way you can protect your children. Do you know that? Yes No
10. More that 57 million doses of HPV vaccine have been distributed to date and all reports note their usefulness – would your decision be to vaccinate your children? Yes No
11. Would you vaccinate your children if it were possible to do so free of charge in Serbia? Yes No
12. Would you decide to vaccinate your children even if the price were €300 per treatment (three are needed)? Yes No
13. Would you decide to vaccinate your children if the price were lower? Yes No
14. Do you think that the price for HPV vaccination in Serbia is too high? Yes No
15. In your opinion, which price level for the HPV vaccine is too high?

- 20,000–30,000 RSD
- 10,000–20,000 RSD
- 5,000–10,000 RSD
- 1,000–5,000 RSD
- < 1,000 RSD

tion of the HPV vaccination decision-maker. Sampling was conducted without the probability, and the sample elements were selected based on the researchers' own estimates. The research sample was an intentional pattern; it represents a basic set, which in turn represents the optimal model used in this kind of pilot studies.

The research was done in accord with standards of the institutional committee on ethics.

RESULTS

As much as 62% (124) of the respondents responded affirmatively to the question, *Do you know what HPV is?* Only 14.5% (29) of those surveyed knew that HPV is the most communicable disease in the world; 34.5% of the respondents answered affirmatively to the question, *Do you know that HPV virus is a direct cause of certain carcinogens?* However, only 15% (30) of the respondents knew that HPV can cause cervical, vaginal, and vulvar cancer in women, penis cancer in men, and anus and mouth or throat cancer, as well as colorectal cancer in both sexes; 22% (44) of the questioned parents knew that HVP is the main cause of condyloma in men and women.

With the knowledge that many years of scientific research have confirmed that HPV vaccine is very effective against the HPV types that cause cervical and conjunctival cancer, when asked if they would decide to vaccinate their child, as many as 94% (188) of the respondents answered affirmatively. Figure 2 shows different parameters that influence parents' decision to vaccinate their children.

With the knowledge that more than 57 million doses of HVP vaccine have been distributed to date without serious safety omissions, to the question whether they would

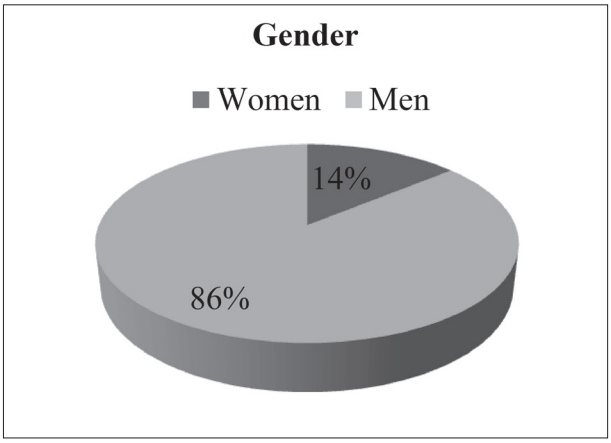

Figure 1. Gender structure of respondents

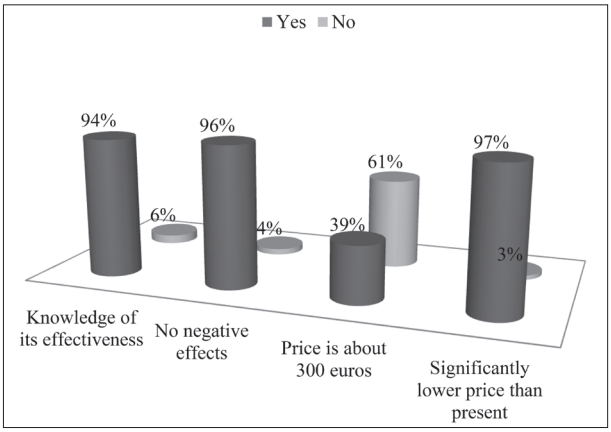

Figure 2. Decisions based on different parameters

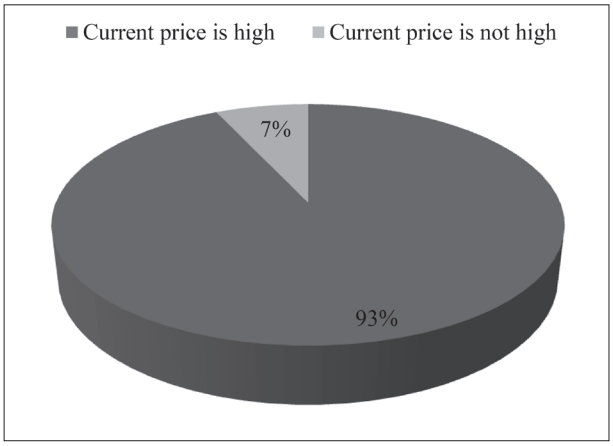

Figure 3. The percentage of respondents who believe that the current market price of HPV vaccines in Serbia is high

Table 1. Opinions of the respondents on the acceptability of the HPV vaccine price

Offered price	n	%
20,000–30,000 RSD	1	0.5
10,000–20,000 RSD	3	1.5
5,000–10,000 RSD	15	7.5
1,000–5,000 RSD	61	30.5
< 1,000 RSD	120	60

decide to vaccinate their child, as many as 96.5% (193) of the respondents answered affirmatively. The same number of respondents would decide to vaccinate their child if there was a possibility of free HPV vaccination in Serbia.

The price of an HPV vaccine of about €300 (three necessary doses) inevitably impacts the decision on vaccinating one's child against the HPV virus; only 39% (78) of the parents decided in favor of the vaccination, which indicates that the price is too high and economically unacceptable, despite the parents' desire to vaccinate their children. As the confirmation of financial inability there was the question, *Would you have decided in favor of vaccination earlier if the price was significantly reduced from the existing one?*, to which 97.5% (195) of the respondents answered affirmatively. At the same time, as much as 93.5% (187) of the respondents considered that the current market price of HPV vaccines in Serbia is too high.

Table 1 presents different prices for the HVP vaccine and the percentages of participants which would accept different levels of prices for the vaccination of their child.

DISCUSSION

Aside from the significance of the HPV vaccine itself, vaccination in Serbia has only recently been applied. Also, to the best of our knowledge, a vaccination campaign against HPV has not been done in Serbia to date.

Our results suggest that knowledge on the importance of vaccination against HPV contributes to the willingness of parents to vaccinate their children. This is in accordance with other authors that recognize the importance of conducting campaigns with the main objective to raise awareness and readiness of parents to vaccinate their children [8]. For example, the major promoter of the HPV vaccination in Canada was the Society of Obstetricians and Gynecologists of Canada [8]. They used a website (hpvinfo.ca) as the main channel for communication in the HPV vaccination campaign. Throughout the website they provided all the necessary information to publicize the importance of HPV vaccination.

Similarly, our results evidenced that the cost of the vaccination process is one of the main obstacles for HPV vaccination in Serbia. Similar findings were reached by other authors, who concluded that the strongest influence on the acceptability of vaccination, besides the type of vaccine, is its cost [9, 10].

Some limitations of the present study should be acknowledged. First, we observed parents that vaccinated, and those who did not vaccinate their children together. Splitting those two samples would probably be useful to find out which were the main drivers that lead some parents to vaccinate their children, in addition to determining obstacles, such as the absence of knowledge and high costs. Also, we did not analyze cultural and social factors that could play a very important role in making a decision on vaccination.

CONCLUSION

HPV vaccine provides highly effective protection against cervical cancer and other HPV-induced diseases. The aim of the HPV vaccination campaign is to significantly reduce the incidence of malignancies caused by HPV infection through the promotion of public awareness of the possibilities, significance, and safety of prevention of the disease through immunization.

The basic principle of communication that is pursued through social networks is continuity. The advantage is a direct channel of communication with clearly defined target groups, with properly created messages. Campaigns implemented in this way give a detailed insight into the ratio of invested resources and achieved results. The campaign focuses on developing awareness of the need for vaccination against HPV viruses, cancer prevention, including the spread of information through social networks such as Facebook, Tweeter, and YouTube to target populations.

The strategy of social marketing on the internet in preventive health care is an important segment of the overall communication of health care to the public. The specificity of the activity requires the authenticity of the approach, as well as the need for promotional campaigns and public advocacy of certain values in order to improve health.

Conflict of interest: None declared.

REFERENCES

1. US National Cancer Institute [Internet]. 2017. Available from: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-vaccine-fact-sheet.
2. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings. J Infect Dis. 2010; 202(12):1789–99.
3. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer. 2009; 45(15):2632–9.
4. Melkert PWJ, van den Brule AJC, Risse EKJ, van Diest PJ, Meijer CJLM, Walboomers JMM, et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. Int J Cancer. 1993; 53(6):919–23.
5. Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189(1):12–9.
6. Vergote I, Van Der Zee AGJ, Kesic V, Sert B, Robova H, Rob L, et al. ESGO statement on cervical cancer vaccination: August 2007. International Journal of Gynecological Cancer. 2007; 17(6):1183–5.
7. Health 2020. A European Policy Framework for Action at All Levels of Government and Society for Health and Wellbeing. [Internet]. World Health Organization. 2012. Available from: http://www.zdravlje.gov.rs/downloads/2012/Decembar/WHO2020.pdf
8. Connell E, Hunt A. The HPV vaccination campaign: A project of moral regulation in an era of biopolitics. Can J Sociol. 2010; 35(1):63–82.
9. Zimet GD, Blythe MJ, Fortenberry JD. Vaccine characteristics and acceptability of HIV immunization among adolescents. Int J STD AIDS. 2000; 11(3):143–9.
10. Lazcano-Ponce E, Rivera L, Arillo-Santillán E, Salmerón J, Hernández-Avila M, Muñoz N. Acceptability of a human papillomavirus (HPV) trial vaccine among mothers of adolescents in Cuernavaca, Mexico. Arch Med Res. 2001; 32(3):243–7.

Креирање друштвене маркетиншке стратегије на интернету у оквиру превентивне здравствене заштите – кампања вакцинације против хуманог папилома вируса

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САЖЕТАК

Увод/Циљ Основни циљ овог чланка је да се развије основа за креирање стратегије маркетинга на интернету у превентивној здравственој заштити кроз истраживање и кампању за вакцинацију против хуманог папилома вируса (ХПВ). Циљ студије је био увођење стратешког приступа социјалног маркетинга на друштвене мреже у кампањи за вакцинацију против хуманог папилома вируса у Србији.

Методе Квантитативно истраживање је спроведено анкетирањем путем интернета, током децембра 2016. године, у које су били укључени родитељи чија су деца кандидати за вакцинацију.

Резултати Добијени резултати истраживања показали су да скоро једна трећина испитаника не зна шта је ХПВ, а скоро исти број испитаника зна да ХПВ узрокује рак. Поред тога, само 14,5% испитаника зна да је ХПВ болест која се најчешће преноси у свету. Уз одговарајући ниво свести о безбедности вакцине, 97% испитаника би одлучило да вакцинише своју децу. Вакцинацију може да приушти само 39% родитеља, мада на промену њиховог мишљења утиче информација о цени ХПВ вакцине. Сходно томе, 97,5% испитаника би се определило за вакцинацију у случају да је бесплатно.

Закључак На основу резултата и поређења са најбољим праксама других земаља, предложили смо маркетиншку стратегију преко друштвених мрежа. Кампања се фокусира на подизање свести о потреби вакцинације против ХПВ-а и превенцију карцинома, укључујући ширење информација циљној популацији путем друштвених мрежа.

Кључне речи: хумани папилома вирус; вакцинација; превенција; социјални маркетинг; стратешки маркетинг на интернету

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SHORT COMMUNICATION / КРАТКО САОПШТЕЊЕ

Stage of glaucoma damage before surgery

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SUMMARY

Introduction/Objective The aim of study was to collect information about factors related to glaucoma damage at the time of surgery in Novi Sad, Vojvodina province, Serbia.

Methods Retrospective data collection on filtrating procedures of 38 patients with open-angle glaucoma was performed. The study was done at the University Eye Clinic, Clinical center of Vojvodina, Serbia between July 2011 and December 2014. The following variables were collected from a data collection sheet at last visit for each patient: age, sex, best-corrected visual acuity (BCVA), visual field indices (MD and PSD), number of active antiglaucoma drugs, glaucoma type, and surgical procedures.

Results The mean age was 66.21 ± 17.92 years. Among patients, 57% were female and 43% were male. Primary open angle glaucoma (POAG) was found in 60% (23/38), pseudoexfoliative glaucoma (XFG) in 37% (14/38) of patients. The median number of active antiglaucoma drugs was 2.73, ranging 1–4. More than 90% of patients were on two or more medications before surgery. Mean BCVA was 0.64 ± 0.68 and oscillated 0.1–1 (according to Snellen). IOP on last visit before surgery varied 15–42 mmHg (mean IOP 26.11 ± 13.20 mmHg). Visual filed index MD showed minor and highest absolute values from -0.82 – -35.25 dB (mean MD -18 dB ± 19.15). All patients had trabeculectomy with Mitomycin C procedures.

Conclusion Our survey found that the level of damage is advanced in terms of visual field loss. In most patients (52.63%), visual acuity was well preserved. POAG and XFG are the most frequent diagnosis. Women and elderly population were represented in higher number in our study. Level of IOP suggests a trend to make a surgical decision at higher pressure regardless the stage of glaucoma damage.

Keywords: glaucoma; stage; damage; surgery

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness in Europe. There are many risk factors for glaucomatous optic neuropathy, but the two most consistent of which appear to be intraocular pressure (IOP) and age [1]. Controlling IOP has been the primary focus of glaucoma treatment. Increasing age was associated with increasing IOP in most studies [1]. Direct correlation between extent of visual field loss and the level of pre-treatment IOP have been found to be weak for primary open angle glaucoma (POAG) [2, 3]. The probability of developing glaucoma at certain IOP may be different for different types of glaucoma [4]. Stronger correlation between visual field loss and IOP has been seen in pseudoexfoliative glaucoma (XFG) [2].

Major risk factors for glaucoma blindness are the severity of the disease at presentation and life expectancy [5]. A young patient with mild optic nerve damage is at much higher risk of getting blind in his lifetime than older patients, so the treatment must be individualized to the needs of rate of progression. Patients with severe functional loss or younger patients with manifest disease should have more aggressive treatment, including filtration surgery [6, 7].

The goal of glaucoma therapy is to maintain good vision for the patients' lifetime, which will sustain the quality of life [8]. In making the right decision for glaucoma surgery, the surgeon must consider the life expectancy of the patient, disease progression rate and the risk-benefit of the other therapy. The surgeon must weigh the surgical benefit such as the likelihood that the surgery will be successful, and prevent further visual loss against the risks of surgical failure and complications. Surgery should be used more frequently at an earlier stage, rather than as a last resort [9]. What needs to be kept in mind is the fact that vision loss from optic nerve damage is irreversible, while vision loss from a common glaucoma surgery complication can be corrected.

Filtration surgery is indicated when medical therapy fails to provide adequate control of intraocular pressure or when IOP is too high in spite of maximal tolerated glaucoma medication [10].

The aim of our study was to collect information about factors related to glaucoma disease at the time of surgery in the city of Novi Sad, Vojvodina, Serbia.

METHODS

This was an observational, retrospective clinical case-series study. This study was done at the Eye Clinic of the Clinical center of Vojvodina,

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Novi Sad in the period between July 2011 and December 2014, and it was done in accord with standards of the institutional Committee on Ethics. Thirty- eight patients (38 eyes) who underwent filtration glaucoma procedures were analyzed in the study. Selected cases had the diagnosis of any type of open angle glaucoma, either primary or secondary, including POAG, normal tension glaucoma, XFG and pigment dispersion glaucoma.

Surgeons had collected the following variables from each patient on the last visit: age, sex, eye, best corrected visual acuity (BCVA), mean deviation of visual field (MD in dB), pattern standard deviation (PSD), IOP on the last visit (mmHg), number of used antiglaucoma drugs, glaucoma type and surgical procedures. Glaucoma was defined as either visual field defect or glaucomatous changes of the optic nerve head (neural rim loss, disc asymmetry, blood vessel changes, peripapillary atrophy). The visual field evaluation was performed using the Humphrey field analyzer- program 24-2 or 30-2 (Carl Zeiss Meditec AG, Jena, Germany) equipped with STATPAC. MD and PSD data was entered for statistical analysis in absolute values.

The data were coded and entered in a database. Statistical analysis was performed using the Statistical Package for Social Sciences. Standard statistical parameters and methods (descriptive statistics and frequency distribution) were used. Numerical data were presented using mean value and standard deviation (SD).

RESULTS

The mean age of all analyzed patients was 66.21 ± 17.92 years. Among them 57% were women and 43% were men. POAG was found in 60% (23/38), XFG in 37% (14/38), and pigmentary glaucoma in 3% (1/38) of patients. We recorded that all our patients had trabeculectomy with Mitomycin C as filtering procedure. The median number of used antiglaucoma drugs was 2.73, ranging from 1–4. More than 90% of patients were on two or more medications before surgery. 2.63% patients were on one medication, 38.84% were on two medications, 44.73% were on three medication and 15.78% were on four medications. Mean BCVA was 0.64 ± 0.68 and was oscillated from 0.1–1 (according to Snellen). There was 52.63% of patients who had BCVA ≥ 0.8 (Table 1).

IOP on the final visit before surgery varied from 15–42 mmHg (minimum and maximum median values). Mean IOP was 26.10 ± 13.20 mmHg and were higher than 21 mmHg in 29/38 patients (76.31%) (Figure 1).

Visual filed index MD showed minor and highest absolute values from -0.82 to -35.25 dB (mean MD -18 dB ± 19.15). Mean PSD value was 6.99 ± 6.27 and varied from 1.5 to 14.6 (Figure 2).

Table 1. Visual function factors, IOP and preoperative number of drugs

Parameters	Mean ± SD	Range (min–max)
Age (years)	66.21 ± 18.16	36–81
IOP (mmHg)	26.11 ± 13.20	15–42
BCVA	0.64 ± 0.68	0.01–1
MD (dB)	-18.01 ± 19.5	-35.52–(-0.82)
PSD	6.99 ± 6.27	1.5–14.6
Number of active drugs	2.74 ± 1.51	1–4

IOP – intraocular pressure; BCVA – best-corrected visual acuity; MD – mean deviation; PSD – pattern standard deviation

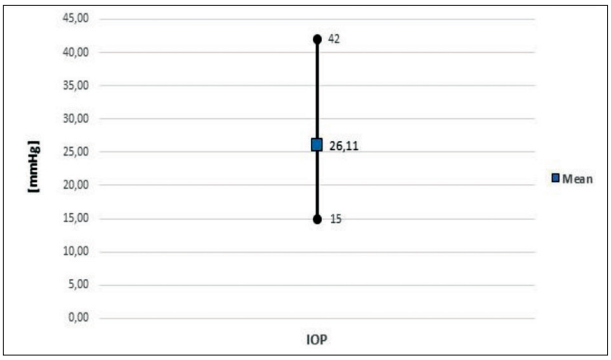

Figure 1. Intraocular pressure before the surgery

IOP – intraocular pressure

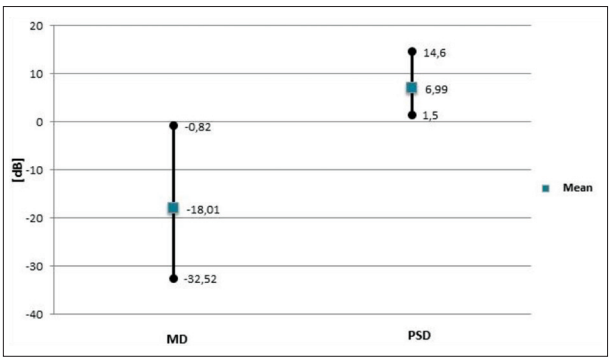

Figure 2. Stage of damage – visual function indices

MD – mean deviation: PSD – pattern standard deviation

DISCUSSION

Our survey found that the level of damage in glaucoma patients before surgery is advanced in terms of visual field loss. Mean visual field index was -18 dB, which is considered advanced visual field loss damage according to Hodapp Classification Staging [11]. Open angle glaucoma was classified as POAG and secondary open angle glaucoma (pseudoexfoliative and pigmentary). Sixty patients had POAG and 37% of patients had XFG. Such a high number of patients with XFG could be explained by the fact that many of them had more progression and more difficulty to control IOP [12]. Surgery is frequently done earlier among XFG patients in contrast to POAG. Nonetheless, studies have shown that the long-term success of trabeculectomy in XFG may be better than that documented with POAG [12].

In our study, women and elderly population were presented in higher number. More than 90% of patients were on two or more medication having in mind that three or more medications are considered the maximal medical antiglaucoma therapy.

Clinical decision making for glaucoma surgery depends on several factors. There are no general recommendations

for glaucoma surgery and for each patient many factors have to be taken into account when choosing a surgical treatment. In patients with POAG, the indications for surgery are the documented visual field and optic nerve damage that threatens the patient's vision, despite the maximal tolerated medication with or without previous laser surgery. IOP that is high enough to place the future health of optic nerve at significant risk is the important factor for surgical indication [13].

Theoretically, it will be more frequently indicated when the disease progresses in the context of maximal medical therapy and uncontrolled IOP, but surgeons can also recommend it either in the progressive patients at risk of vision threatening, despite an apparently well controlled IOP, or when IOP is unacceptable high regardless the functional status. Our survey found that more than 90% of patients were on two or more medication and only 15.78 % of patients were on maximal medical therapy showing that filtration surgery was not the last resort, which is not in correlation with the guidelines [9, 10, 14]. Analysis of IOP level before surgery also showed that when IOP is too high despite the level of visual function loss, surgeons could decide to perform filtering procedures regardless of stage of the damage [15].

The glaucoma staging applications nowadays allows automated, reproducible, and objective classification system for staging glaucoma damage for multiple 24-2 visual functions of Humphrey visual field. Recent publication was a proof of concept that could translate into useful tool to analyze and stage visual functions more objectively [16]. Latest results regarding quality of life in glaucoma patients demonstrate that quality of life is impaired in patients with glaucoma and this alteration is greater the more advanced is glaucoma damage in the best or both eyes [17].

In the last few years, the authors continued to collect new data of the stage of glaucoma damage before the surgery and the study will be updated in near future.

CONCLUSION

Our survey found that the level of damage is advanced in terms of visual field loss. In most patients (52.63%) visual acuity was well preserved. POAG and XFG are the most frequent diagnosis. Women and elderly population were represented in higher number in our study. Level of IOP suggests a trend to make a surgical decision at higher pressure regardless the stage of glaucoma damage.

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This survey was in part presented at EGS meeting in September 2–3, 2011 Malmo, Sweden as retrospective data collection on filtration glaucoma procedures performed in open-angle glaucoma around Europe in 2011.

Conflict of interest: None declared.

REFERENCES

1. Gazzard G, Foster PJ, Devereux JG, Oen F, Chew PT, Khaw PT, et al. Intraocular pressure and visual field loss in primary angle closure and primary open angle glaucoma. Br J Ophthalmol. 2003; 87(6):720–5.
2. Teus MA, Castejon MA, Calvo MA, Pérez-Salaíces P, Marcos A. Intraocular pressure as a risk factor for visual field loss in pseudoexfoliative glaucoma and in primary open-angle glaucoma. Ophthalmology. 1998; 105(12):2225–9.
3. Vogel R, Crick RP, Newson RB, Shipley M, Blackmore H, Bulpitt CJ. Association between intraocular pressure and loss of visual field in chronic simple glaucoma. Br J Ophthalmol. 1990:74(1):3–6.
4. Davanger M, Ringvold A, Bilka S. The probability of having glaucoma at different IOP levels. Acta Ophthalmol. 1991:69(6):565–8.
5. Martus P, Stroux A, Budde WM, Mardin CY, Korth M, Jonas JB. Predictive factors for progressive optic nerve damage in various types of chronic open-angle glaucoma. Am J Ophthalmol. 2005; 139(6):999–1009.
6. The AGIS investigators. The Advance Glaucoma Intervention Study (AGIS): The relationship between control of intraocular pressure and visual field deterioration. Am J Ophthalmol. 2000:130(4):429–40.
7. AGIS investigators. The Advance Glaucoma Intervention Study (AGIS). Baseline risk factors for sustained loss of visual field and visual acuity in patients with advanced glaucoma. Am J Ophthalmol. 2002; 134(4):499–512.
8. Orta AÖ, Öztürker ZK, Erkul SÖ, Bayraktar Ş, Yilmaz OF. The correlation between glaucomatous visual field loss and vision-related quality of life. J Glaucoma. 2015; 24(5):121–7.
9. Watson PG. When to operate in open-angle glaucoma. Eye. 1987; 1:51.
10. Babić N. Fixed combination of glaucoma medications. Srp Arh Cel Lek. 2015; 143(9–10):626–31.
11. European Glaucoma Society. Terminology and Guidelines for Glaucoma. 4th ed. Savona, Italy: PubliComm; 2014. p. 64.
12. Holló G, Katsanos A, Konstas AG. Management of exfoliative glaucoma: challenges and solutions. Clin Ophthalmol. 2015; 9:907–19.
13. Stamper RL, Lieberman MF, Drake MV. Becker-Shaffer's diagnosis and therapy of the glaucomas. 8th ed. Mosby-Elsevier; 2009. P. 462–5.
14. Sethi HS, Naik MP, Saluja K. Role of trabeculectomy in advanced glaucoma: Whether we stand to consider it a bane or a boon today? Int Ophthalmol. 2017; 2019; 39(1):137–43.
15. Sturmer JPE, Faschinger C. Do we perform glaucoma surgery too late? Klin Monbl Augenheilkd. 2018; 235(11):1269–77.
16. Germano RAS, de Moraes CG, Susanna R Jr, Dantas DO, Neto EDS. Evaluation of a Novel Visual Field Analyzer Application for Automated Classification of Glaucoma Severity. J Glaucoma. 2017; 26(6):586–91.
17. Pujol-Carreras O, Anton A, Mora C, Pastor L, Gudiña S, Maull R, et al. Quality of life in glaucoma patients and normal subjects related to the severity of damage in each eye. Arch Soc Esp Oftalmol 2017; 92(11):521–7.

Степен глаукомског оштећења пре хируршке интервенције

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САЖЕТАК

Увод/Циљ Циљ истраживања је био да се прикупе подаци о факторима који су повезани са глаукомским оштећењем код болесника пре хируршке интервенције у Новом Саду (Војводина, Србија).

Методе У ретроспективној студији анализирано је 38 болесника са глаукомом отвореног угла којима је извршена филтрациона операција на Клиници за очне болести Клиничког центра Војводине у периоду од јула 2011. до децембра 2014. За сваког болесника на последњој контроли пре хируршке интервенције прикупљени су следећи подаци: године старости, пол, најбоља коригована видна оштрина, индекси видног поља (*MD* и *PSD*), број лекова у терапији глаукома, врста глаукома и врста интервенције.

Резултати Просечна старост болесника у овој студији износила је 66, 21 ± 17, 92 година. Од укупног броја болесника било је 57% жена и 43% мушкараца. Примарни глауком отвореног угла имало је 60% (23/38), а псеудоексфолијативни глауком 37% (14/38) болесника. Просечан број коришћених антиглаукомских лекова износио је 2,73 и кретао се од 1 до 4. Више од 90% болесника било је на два или више антиглаукомска лека пре оперативног лечења. Просечна најбоља коригована видна оштрина износила је 0,64 ± 0,68 у распону 0,1–1,0 (по Снелену). Интраокуларни притисак на последњем мерењу пре оперативног лечења кретао се 15–42 *mmHg* (просечан интраокуларни притисак је 26,11 ± 13,20 *mmHg*). Индекс видног поља имао је најмање и највеће апсолутне вредности -0,82 *dB* – -35,25 *dB* (просечан *MD* -18,00 ± 19,15). Сви наши болесници имали су трабекулектомију са митомицином Це.

Закључак Наша студија је показала да болесници имају висок степен оштећења по индексу видног поља. Код највећег броја испитаника (52,63%) видна оштрина била је добро очувана. Примарни глауком отвореног угла и псеудоексфолијативни глауком биле су најчешће дијагнозе. У испитаној групи болесника већа је заступљеност жена и особа старије животне доби. Висина интраокуларног притиска сугерише тренд да се одлука о хируршкој интервенцији доноси код његових високих вредности, без обзира на степен оштећења.

Кључне речи: глауком; степен оштећења; хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Recurrent epistaxis as a manifestation of renal cell carcinoma sinonasal metastasis

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SUMMARY

Introduction Renal cell carcinoma is the most frequent infraclavicular primary tumor metastasizing in the sinonasal region, although these metastases are not common. We present an unusual case of recurrent epistaxis as the initial sign of renal carcinoma sinonasal metastasis and discuss the diagnostic and treatment options.

Case outline A 66-year-old patient was admitted to the hospital due to recurrent and severe epistaxis. The patient underwent nephrectomy due to renal cell carcinoma, with no signs of relapse during a three-year follow-up. Nasal endoscopy and computed tomography revealed a large mass in nasal cavity, spreading to the anterior and posterior ethmoid cells, sphenoid sinus, orbit, and anterior cranial fossa. Definite diagnosis of renal cell carcinoma metastasis in sinonasal region was made by a pathologist after biopsy and further radiological examination showed no signs of malignant disease in the abdomen, thorax, or pelvis. Although the patient had received 50 Gy of radiation therapy, the malignant disease was evaluated as progressive with further extension in anterior cranial fossa and maxilla, and the patient died five months after the occurrence of epistaxis.

Conclusion In patients with recurrent epistaxis who also had a history of renal carcinoma, endoscopic finding of tumefaction in the nasal cavity should raise a suspicion of sinonasal metastasis. In such cases, biopsy is mandatory to differentiate a metastasis from primary sinonasal tumors. Histological confirmation should be followed by radiological examination of the abdomen, thorax, and pelvis to evaluate the possibility of renal cell carcinoma recurrence or metastatic dissemination elsewhere.

Keywords: epistaxis; renal cell carcinoma; neoplasm metastasis; nasal cavity

INTRODUCTION

Nasal cavity and paranasal sinuses are very rare sites of metastatic disease. Renal cell carcinoma (RCC) is the most common malignant renal tumor with unpredictable course that develops extranodal metastases of the head and neck in 1.1% of cases [1]. It is reported to be the most frequent infraclavicular primary tumor metastasizing in the sinonasal region, although these metastases are not common [2].

We present an unusual case of recurrent epistaxis as the initial sign of RCC sinonasal metastasis and discuss the diagnostic and treatment options.

The patient's written consent was obtained and publication conforms to the ethical standards.

CASE REPORT

A 66-year-old male was admitted to the hospital due to recurrent and severe epistaxis. The patient had a medical history of hypertension and cardiac arrhythmia regulated with standard medications prescribed by the cardiologist and pacemaker implanted four years earlier. The patient underwent nephrectomy due to RCC, with no signs of relapse during a three-year follow-up. Also, he had sigmoid colon carcinoma resected 13 years earlier, with no signs of recurrence.

The initial laboratory testing excluded a coagulopathy or high blood pressure as a direct cause of bleeding (prothrombin time 10.7 seconds, activated partial thromboplastin time 31.7 seconds, international normalized ratio 1.1, platelet count 210×10^3/mL, blood pressure 110/80 mmHg).

During an endoscopic examination, a large hypervascular outgrowth in the nasal cavity was determined as the source of the bleeding. Computed tomography (CT) scan showed a soft-tissue density mass in the nasal cavity, spreading to the anterior and posterior ethmoid cells, the sphenoid sinus, the orbit, and the anterior cranial fossa (Figure 1). Despite the CT scan finding, the patient had no visual impairments or neurological deficits, except hyposmia. During hospital treatment, the patient complained of frontal headaches, which were treated with standard analgesic therapy.

Definite diagnosis of RCC metastasis in the sinonasal region was made by a pathologist after an endoscopic biopsy (Figure 2). Hematoxylin and eosin staining showed a tumor

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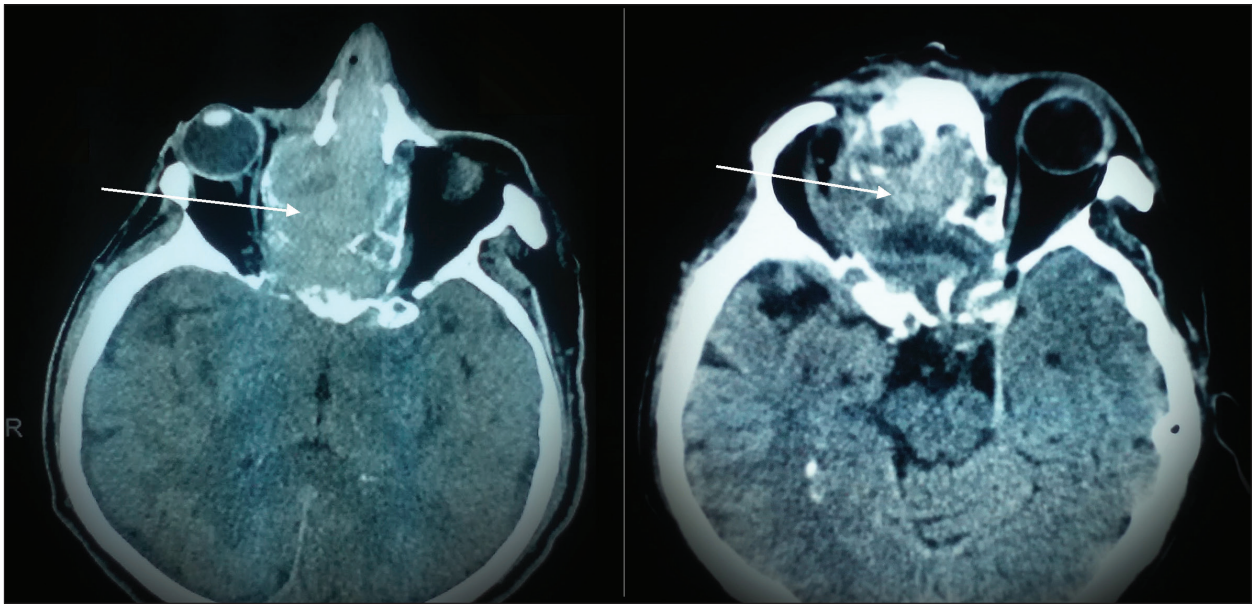

Figure 1. The 66-year-old male patient with recurrent and severe epistaxis and a history of colon carcinoma resection and nephrectomy due to renal cell carcinoma; CT scan of the paranasal sinuses: axial views; arrows point to a renal cell carcinoma sinonasal metastasis with orbital and anterior cranial fossa extension

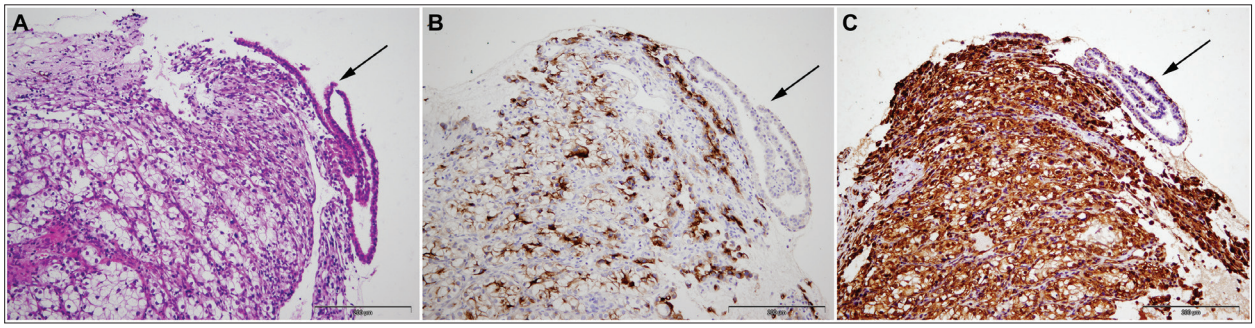

Figure 2. Histopathological findings after endoscopic biopsy of a sinonasal tumor in the 66-year-old male patient; A) the tumor is under the strip of flattened pseudostratified epithelium (arrow); the tumor is composed of large clear cells organized in acini (H&E); B) the tumor is positive for renal cell carcinoma antigen; C) the tumor is positive for vimentin; pseudostratified epithelium is negative (arrow)

composed of large clear cells organized in acini. The cells showed immunohistochemical positivity for the RCC antigen, vimentin, and CD10, but were negative for CK7 and CK20.

Further radiological examination with CT showed no signs of malignant disease in the abdomen, the thorax, or the pelvis. The neck ultrasound finding was normal, with no enlarged lymph nodes or signs of neoplastic disease in the thyroid gland.

Although the patient had received 50 Gy of radiation therapy, malignant disease was evaluated as progressive with further extension in the anterior cranial fossa and the maxilla. The patient with incurable RCC sinonasal metastasis died five months after the epistaxis appearance.

DISCUSSION

Nasal secretion, stuffy nose, and epistaxis are very common complaints in otorhinolaryngology practice. In such cases, detection of hypervascular tumefaction in the nasal cavity found during clinical examination should raise suspicion about primary sinonasal tumor, such as hemangiomas, hemangiopericytomas, adenocarcinomas, or melanomas. Infrequently, those patients are diagnosed with metastatic sinonasal tumor spreading from distant primary sites. Primary and metastatic sinonasal tumors are difficult to differentiate by clinical and radiological examination – therefore, biopsy is recommended.

Sinonasal metastases are quite rare; however, RCC is the most common malignancy that metastasizes to this area [3]. Common sites of RCC metastases are the lungs, the liver, the adrenal glands, the brain, and bones. Most of the patients develop multiple RCC metastases, such as in the lung and the liver; on the other hand, sinonasal metastases are commonly solitary.

Hematogenous spread of RCC tumor cells to the sinonasal region is carried out by two routes; one is responsible for the dissemination through the inferior vena cava, the heart, the lungs, and maxillary artery, and the other leads to the head and neck region via Batson's paravertebral venous plexus, bypassing the lungs. Therefore, the caval route could be responsible for the concurrent lung or brain metastases, and the retrograde venous route could

explain uncommon presentation of sinonasal metastasis without evidence of malignant tumor elsewhere. Another explanation for solitary sinonasal metastasis of RCC could be lymphatic spread of tumor cells via the thoracic duct.

Today, RCC is often asymptomatic and is generally detected incidentally. One third of newly diagnosed RCC patients have a distant metastasis as the initial presentation; another third of RCC patients develop a metastasis during the follow-up [4]. In our case, the patient underwent curative nephrectomy three years before the appearance of recurrent nasal bleeding and had been disease-free during the regular follow-up. Radiological examination showed solitary sinonasal metastasis with no metastatic spreading elsewhere. The long latency interval may be attributed to the slow-growing characteristic of RCC and the fact that RCC is under the influence of the host immunity. Were there any metastasis elsewhere undetected by the radiological examination or did RCC develop a solitary sinonasal slow-progressive metastasis is the question that needs to be addressed.

The common presentation of RCC sinonasal metastasis comprises nasal obstruction, swelling, and pain. Approximately 46% of these patients complained of recurrent epistaxis due to highly vascular nature of RCC and its metastasis [5]. Identification of von Hippel–Lindau tumor suppressor (pVHL) considerably improved the understanding of RCC molecular biology. pVHL is a component of E3 ubiquitin ligase complex that targets α units of hypoxia-inducible factors (HIF-1, HIF-2) for proteasomal degradation in the environment with a normal oxygen concentration. In hypoxic conditions, HIF-1α is stabilized and induces the transcription of a number of downstream genes involved in pathogenesis of head and neck squamous cell carcinoma. HIF-1α significantly contributes to carcinogenesis by inducing angiogenesis through the synthesis of vascular endothelial growth factor (VEGF). HIF is known to be upregulated by the VHL gene, whose functional loss is identified in the majority of clear cell RCCs. This sequence of events is responsible for increased vascularity of RCC sinonasal metastases and epistaxis as a major sign of the disease.

Histopathological confirmation of sinonasal metastatic RCC should be followed by the evaluation of the possible recurrence of the primary tumor or distant spreading elsewhere. Positron emission tomography – computed tomography (PET-CT) has high sensitivity and specificity in detecting RCC recurrence and results are in correspondence with conventional radiological examination with CT and magnetic resonance imaging [6]. On the other hand, PET-CT is more powerful in detecting early metastatic disease, especially in bones and muscles [7]. High level of false positive results due to inflammation or scaring should raise caution with clinicians during RCC evaluation on PET-CT.

Metastatic renal carcinoma has a poor prognosis due to chemotherapy and radiotherapy resistance and silent growth. Surgical treatment is considered the optimal choice for patients with single resectable sinonasal metastasis. Five-year survival of 35% is reported with such patients after radical excision and nephrectomy [5]. Treatment modalities such as radiotherapy, chemotherapy, or immunotherapy are reserved for patients with unresectable metastatic disease. In the last decade, numerous molecular-targeted agents were approved with positive impact on survival of patients with metastatic RCC [8]. Antiangiogenic therapy with VEGF inhibitors is considered the first-line of targeted therapy, comprising a great variety of novel agents [9]. Generally, chemotherapy is used with patients who did not respond to immunotherapy. Patients with metastasis in multiple organs have a very poor prognosis, with five-year survival below 7% [10]. Our patient had a nonresectable metastasis and radiotherapy did not achieve any benefit, which can be explained by tumor biology and volume.

In patients with recurrent epistaxis that also had a history of renal carcinoma, endoscopic finding of tumefaction in the nasal cavity should raise suspicion of RCC sinonasal metastasis. In such cases, biopsy is mandatory to differentiate a metastasis from primary sinonasal tumors. Histological confirmation should be followed by radiological examination of the abdomen, the thorax, and the pelvis to evaluate the possibility of RCC recurrence or metastatic dissemination elsewhere.

Conflict of interest: None declared.

REFERENCES

1. Franzen AM, Schneider C, Lebentrau S, Gunzel T. Extranodal Metastases of Renal Cell Carcinoma to the Head and Neck: A Diagnostic Challenge. Laryngorhinootologie. 2015; 94(11):745–8.
2. Lee SM, Kim YM, Kim BM. Epistaxis as the First Manifestation of Silent Renal Cell Carcinoma: A Case Report with Relevant Literature Review. Iran J Radiol. 2016; 13(1):e31208.
3. Mahajan R, Mayappa N, Prashanth V. Metastatic Renal Cell Carcinoma Presenting as Nasal Mass: Case Report and Review of Literature. Indian J Otolaryngol Head Neck Surg. 2016; 68(3):374–6.
4. Bastier PL, Dunion D, de Bonnecaze G, Serrano E, de Gabory L. Renal cell carcinoma metastatic to sinonasal cavity: A review and report of 8 cases. Ear Nose Throat J. 2018; 97(9):E6–12.
5. Evgeniou E, Menon KR, Jones GL, Whittet H, Williams W. Renal cell carcinoma metastasis to the paranasal sinuses and orbit. BMJ Case Rep. 2012; 2012.
6. Park JW, Jo MK, Lee HM. Significance of 18F-fluorodeoxyglucose positron-emission tomography/computed tomography for the postoperative surveillance of advanced renal cell carcinoma. BJU Int. 2009; 103(5):615–9.
7. Liu Y. The Place of FDG PET/CT in Renal Cell Carcinoma: Value and Limitations. Front Oncol. 2016; 6: 201.
8. Cavaliere C, D'Aniello C, Pepa CD, Pisconti S, Berretta M, Facchini G. Current and emerging treatments for metastatic renal cell carcinoma. Curr Cancer Drug Targets. 2018; 18(5):468–79.
9. Kuusk T, Albiges L, Escudier B, Grivas N, Haanen J, Powles T, et al. Antiangiogenic therapy combined with immune checkpoint blockade in renal cancer. Angiogenesis. 2017; 20(2):205–15.
10. Ziari M, Shen S, Amato RJ, The BS. Metastatic renal cell carcinoma to the nose and ethmoid sinus. Urology. 2006; 67(1):199.

Понављајуће крварење из носа као манифестација синоназалне метастазе карцинома бубрега

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САЖЕТАК

Увод Карцином реналних ћелија је најчешћи инфраклавикуларни тумор који метастазира у синоназални регион, мада ове метастазе нису толико честе. Представљамо необичан случај болесника код кога је крварење из носа било први знак синоназалне метастазе реналног карцинома и дискутујемо о дијагностичким и терапијским могућностима.

Приказ болесника Шездесет шестогодишњи болесник је примљен на болничко лечење због понављајућег, интензивног крварења из носа. Болеснику је претходно због карцинома одстрањен бубрег и током трогодишњег праћења није имао знакове релапса малигне болести. Ендоскопским прегледом и компјутеризованом томографијом утврђен је велики израштај у носној шупљини, који се шири у предње и задње етмоидне ћелије, сфеноидни синус, очну дупљу и предњу мождану јаму. Биопсијом је постављена дијагноза метастазе карцинома бубрега, а даља радиолошка испитивања су искључила постојање релапса малигне болести у малој карлици, абдомену или грудном кошу. Иако је болесник примио радиотерапију, тумор је испољио даљу прогресију у предњој можданој јами и максили, а болесник је преминуо пет месеци после појаве крварења из носа.

Закључак Код болесника са понављајућим крварењем из носа који у личној анамнези имају податак о карциному бубрега и код којих је ендоскопским прегледом виђен тумефакт у носној шупљини, требало би посумњати на могућност синоназалне метастазе. У таквим случајевима обавезна је биоспија како би се разликовао примарни тумор од метастазе. После патохистолошке потврде метастазе карцинома бубрега потребно је спровести радиолошко испитивање мале карлице, абдомена и грудног коша како би се искључила даља дисеминација малигне болести.

Кључне речи: епистакса; карцином бубрега; метастаза тумора; носна шупљина

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A case of a three-month-old female infant with incarcerated femoral hernia

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SUMMARY
Introduction Femoral hernia in children is very rare and it appears in 0.2% of all hernias during childhood. It is three times more frequent in girls. The aim of this paper is to present a female infant with incarcerated femoral hernia.
Case outline A female infant aged three months was hospitalized with classical clinical signs of incarcerated left femoral hernia. After preoperative preparation, a transversal inguinal incision was performed on the left side and an incarcerated femoral hernia was confirmed with a torn and gangrenous left ovary and fallopian tube. Following adnexectomy, the hernia sac was ligated and resected and hernioplasty was made by the reconstruction of the Cooper's ligament.
Conclusion A delayed surgical intervention for incarcerated femoral hernia in a female can result in a loss of the ovary and the fallopian tube, which may lead to serious consequences later in life.
Keywords: femoral hernia; incarceration; ovary and fallopian tube

INTRODUCTION

According to McVay and Savage [1], hernias represent a defect in the continuity of the normal musculo-aponeurotical and fascial abdominal wall. They can be either congenital or acquired, with a protrusion of the abdominal cavity contents through the openings of the abdominal wall. Persistent patent *processus vaginalis* is presented by the peritoneal turnup which goes from the inguinal region to the scrotum or labium [2]. Femoral and direct inguinal hernias in children are very rare.

Femoral hernia is the type of hernia in which a protrusion engages the femoral ring and *fascia transversalis*, penetrating into *regio cruralis* through relatively small femoral openings.

Femoral canal has got the shape of a cone with two holes. The first one, femoral ring, which is the entry part of the femoral canal, and the second one, much smaller, is the lower femoral hernia opening or saphenous opening, which stands a few centimeters lower of inguinal ligament. The saphenous opening is the exit part. Accordingly, femoral hernia is diagnosed only when the contents of the femoral hernia pass through the canal. In normal conditions, alongside the connective tissues are some small lymph nodes (Cloquet–Rosenmuller). Above the distal femoral hernia hole there is *fascia cribriformis*.

The anatomical variation of the narrow posterior inguinal wall attachment on to the pectineal ligament, resulting in an enlarged femoral ring, may be the primary etiological factor, according to Tasche [3]. The increased intraabdominal pressure enables the herniation.

Incarcerated femoral hernias are more frequent in comparison with direct and indirect inguinal hernias. It is important to diagnose this state in an appropriate time because complications can endanger a patient's life.

CASE REPORT

A girl of three months was admitted for immediate treatment at the local health center. Upon admission, the child showed clinical signs of a very ill patient. The abdomen was lightly painful in the lower parts. In the left inguinal area, there was a nut-sized lump with erythematous skin. The symptoms began five days previously with irritability and crying. She arrived to her doctor after three days due to objective reasons. The symptoms did not improve and she was hospitalized. According to the history and clinical work-up (Table 1), a surgical intervention under general anesthesia was indicated. A transversal inguinal incision was made. The

Table 1. Values of blood test results on admission

Blood element	Value
RBC	3.7×10^{12}/l
HGB	113 g/l
HCT	0.32 l/l
WBC	27×10^{9}/l
GRA	83%
LYM	12%
MON	4.5%
EO	0.5%

RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; WBC – white blood cells; GRA – granulocytes; LYM – lymphocytes; MON – monocytes; EO – eosinophils

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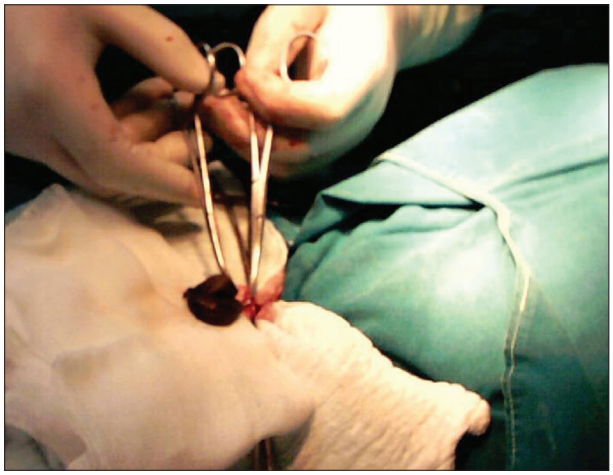

Figure 1. Femoral hernia with a gangrenous ovary

Figure 2. Adnexectomy on the left side

twisted and gangrenous ovary and fallopian tube were found in the hernial sac (Figure 1). Left adnexectomy was performed (Figure 2). The hernial sac was ligated and resected. Hernioplasty was made by the reconstruction of the Cooper's ligament and the wound was closed by layers. The operation was uneventful and postoperative period was without complications.

DISCUSSION

Femoral hernias are very rare in children. A review by Fonkalsrud et al. [4] of 5,452 cases, and Burke's [5] review of 4,567 patients showed only 21 (0.2%) patients with femoral hernias. The age of the patients was from six weeks to 13 years. Five patients had strangulated femoral hernias [4–7]. They are more frequent in girls than in boys (3:1) [8].

In our case, we had incarcerated femoral hernia on the left side, whose content was fallopian tube and ovary. In the literature it is described as a rare occurrence [9, 10].

In the modern surgical practice, there is a tendency to diagnose every type of hernia as soon as possible, which makes the intervention easier and provides better postoperative results.

Incarcerated hernias pose serious complications. Usually, incarcerated organs are the small intestine, the colon, the appendix, the omentum, the ovaries, and the fallopian tubes. In incarcerated hernias, manual reposition by taxis can be dangerous, so immediate surgical interventions are suggested.

The diagnosis, treatment, and the prognosis of incarcerated hernias depend on the degree of the pathological changes in venous and arterial blood flow of incarcerated organs [11]. If this process continues for a sufficient period of time, which can vary from hours to days, herniated content becomes gangrenous and necrotic.

The optimal chosen method for the type of hernia is a precondition for optimal postoperative results. The method should be maximally physiological, and the disruption of the functional integrity of the back wall of the inguinal canal should be as little as possible. Our choice was the method which, in the presented case, provides the best result. A good result is characterized by small postoperative morbidity and low percentage of recurrence.

We used the McVay's repair method, which is usually the most common treatment for femoral hernias in children, and some surgeons state that it gives fewer recurrences [12, 8]. In femoral hernia surgery, it is essential to ligate the sac, manage the posterior wall of the inguinal canal, and suture the femoral ring regardless of which method is used [13, 14].

In order to avoid complications which can lead to strangulated hernias, adequate checkup is needed, as well as the correct and opportune diagnosis as an indication for surgical treatment. In the presented case, the situation was perceived and the baby girl of three months was brought to the surgeon too late. During the operation, the most appropriate surgical method for a twisted and gangrenous ovary and fallopian tube was adnexectomy.

Even with serious consequences for the patient, we expect her to retain adequate reproductive possibilities with preserved functionality.

Conflict of interest: None declared.

REFERENCES

1. McVay CB, Savage LE. Etiology of Femoral Hernia. Annals of Surgery. 1961; 154(Suppl 6):25–32.
2. Applebaum H, Bautista N, Cymerman J. Alternative method for repair of the difficult infant hernia. J Pediatr Surg. 2000; 35(2):331–3.
3. Tasche LW. Etiology of femoral hernia. Arch Surg. 1932; 25:749–82.
4. Fonkalsrud EW, de Lorimer AA, Clatworthy HW Sr. Femoral and direct inguinal hernias in infants and children. J Am Med Assoc. 1965; 192:101–3.
5. Burke J. Femoral hernia in childhood. Ann Surg. 1967; 166(2):287–9.
6. Al-Shanafey S, Giacomantonio M. Femoral hernia in children. J Pediatr Surg. 1999; 34:1104–6.

7. Radcliffe G, Stringer MD. Reappraisal of femoral hernia in children. Br J Surg. 1997; 84(1):58–60.
8. Tam PK, Lister J. Femoral hernia in children. Arch Surg. 1984; 119:1161–4.
9. Shepler NB, Smith CW. Femoral hernia in infancy. Am J Surg. 1949; 49:375–7.
10. Cloquet JG. Nouvelles recherches sur la hernies del'ovarie [Cited in Puech, A]. Ann Gyne´col (Paris). 1878-1879; 10:321–38.
11. Rajpurt A, Gauderer MWL, Hack M. Inguinal hernias in very low birth weight infants: Incidence and timing of repair. J Pediatr Surg. 1992; 27(10):1322–4.
12. Marshall DG. Femoral hernias in children. J Pediatr Surg. 1983; 18:160–2.
13. Tsushimi T, Takahashia T, Gohraa H, Fukudaa S, Furukawaa S, Odaet T, et al. A case of incarcerated femoral hernia in an infant. J Pediatr Surg. 2005; 40(3):581–3.
14. Sorelli PG, El-Masry NS, Garrett WV. Open femoral hernia repair: one skin incision for all. WJES. 2009; 4:44.

Приказ тромесечног женског одојчета са инкарцерираном феморалном хернијом

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САЖЕТАК

Увод Феморална хернија код деце је изузетно ретка и јавља се у око 0,2% свих хернија дечје доби. Троструко је чешћа код деце женског пола. Приказујемо женско одојче са инкарцерираном феморалном хернијом.

Приказ болесника Женско одојче узраста три месеца хоспитализовано је са класичним клиничким знацима инкарцериране феморалне хернije са леве стране. После преоперативне припреме направљен је попречни рез ингвинално лево, где је нађена укљештена феморална хернија са торквираним и гангренозно измењеним оваријумом и тубом утерином. После учињене аднексектомије, килна кеса је подвезана и ресецирана и направљена је пластика канала реконструкцијом Куперовог лигамента.

Закључак Прекасна хируршка интервенција је резултирала губитком левог аднекса, што може оставити озбиљне последице на њен даљи живот.

Кључне речи: феморална хернија; инкарцерација; оваријум

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Calcification of a cervical intervertebral disc in a child – a case report and review of literature

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SUMMARY

Introduction We report a case of calcification of a cervical intervertebral disk in a child. This is a rare condition, and has been described in about 400 cases worldwide. Children affected by it present with the onset of pain, muscle spasm, and the presence of calcification of a intervertebral disk on radiography. Our objective was to present a case of sudden onset of pain in the neck and torticollis.

Case outline In our case, the condition was diagnosed after trauma, presented with neck pain and spasm of the right sternocleidomastoid. Initial neck radiography was done, and after identifying the calcification in front of C4 and C5 vertebral bodies, CT analysis was conducted. When it was concluded that there is no compression on spine nerve roots, conservative course of treatment was followed. The child had full regression of symptoms after two weeks.

Conclusion Emergency personnel should bear in mind that, even though radiographical finding of a calcification shadow in front of the spine may raise concern, the nature of this disorder is benign in most cases, and responds very well to conservative treatment.

Keywords: calcification; intervertebral disk; neck pain; torticollis

INTRODUCTION

Juvenile calcification of an intervertebral disc is an infrequent condition, benign and self-limiting in character, primarily affecting *nucleus pulposus*. Luschka described the first case of intervertebral disc calcification on anatomic dissection in 1858, and Beneke first demonstrated this condition radiographically in 1897 [1, 2, 3]. This disorder in children was first discovered by Baron [4] in 1924, and others found it in more than 400 cases to date.

Our objective was to present a case of a sudden onset of pain in the neck and torticollis.

CASE REPORT

The patient, a six-year-old girl, was brought to the clinic because of a sudden onset of pain in the neck and torticollis. After examining the patient history, we found that she fell from her bicycle two days earlier and was treated for subacute rhinitis for the previous two weeks.

Clinical examination revealed tilting of the head to the right side and upwards, consistent with a muscle spasm of the sternocleidomastoid muscles. There was also asymmetry of the shoulders and elevated tonus of the posterior neck and shoulder muscles. Active range of motion for the cervical part of the spine was significantly decreased: flexion 25°, extension 0°, right and left lateral flexion 10°, 60° right rotation, 40° left rotation. Postural dysfunction and thoracolumbar scoliosis was also noted. Pain was provoked by terminal movement and palpation of the posterior side of the neck bilaterally. No motor neurological deficits were present and no sensorimotor neurological deficits were noted (tests for gross motor strength, gross motor functions, fine motor functions, and simple sensory skill were performed).

Because of positive trauma heteroanamnesis, plain radiography of the cervical spine was taken. We discovered a calcified lesion between C4 and C5 vertebrae, which we first attributed to trauma (Figure 1). On the following day, computerized tomography (CT) scan was done without the contrast (Figure 2). The results of the analysis were as follows: oval hyper dense lesion (density of calcium) in the intervertebral space at the level of C4/C5 vertebrae, centrally positioned, approximately 5.5 × 5.5 mm, with nearby punctiform calcifications. An oval lesion was situated in the intervertebral disc, its anterior aspect and punctiform calcification were in the lateral aspect of the disc. There was no penetration in the spinal canal and no signs of compression on spinal nerves were present. The cervical part of the spinal canal was con-

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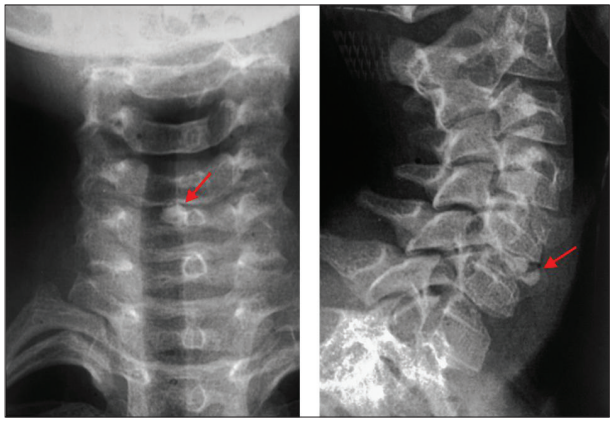

Figure 1. Radiography of the cervical spine; calcified lesion between C4 and C5 vertebrae

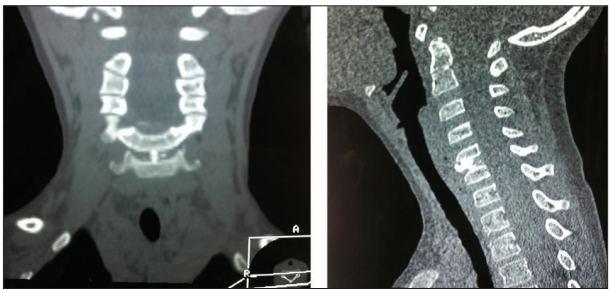

Figure 2. Computerized tomography scan of cervical spine without contrast; calcified lesion between C4 and C5 vertebrae

sistent and with no pathological changes. There were signs of sclerosation of the inferior plateau of the C4 vertebral body and on the superior plateau of the C5 vertebral body.

Since there were no neurological symptoms and CT did not show any pathological changes related to the spinal canal, magnetic resonance imaging (MRI) was not indicated.

The patient was treated conservatively, analgesics were prescribed, soft cervical collar (Schantz) was placed for seven days, and after the initial management the patient was referred to a physiotherapist for further treatment. All symptoms were gone after two weeks.

DISCUSSION

Juvenile calcification of an intervertebral disc is an uncommon childhood condition characterized by neck pain, torticollis, and calcification of the intervertebral disk. Most of the patients were aged between five and 12 years, predominantly male (male:female = 8:5), though some studies suggest a different ratio of male to female (1:1) [5, 6]. These results should be taken carefully, as studies were done on small series of patients [7].

Calcification could be found on any level of the spine and there may be multiple level involvements in 30–40% of cases [5, 6, 8, 9, 10]. The most affected part of the spine is the cervical one. The *nucleus pulposus* and *annulus fibrosus* can be calcified, but in the majority of the cases it is the *nucleus pulposus*. When calcifications are found in the cervical spine, its lower part is more frequently involved and almost all patients are symptomatic [7, 11]. The onset is usually acute, and patients are often referred to emergency room for evaluation of a possibly more serious condition.

Muscle pain was the major symptom of the condition, and it is found in about 70% of patients, followed by sensorimotor disturbance (focal weakness, sensory loss, compressive myelopathy) and fever [7]. Dysphagia has been reported in a patient with an anterior disk protrusion and is attributed to the formation of a retropharyngeal edema due to tissue irritation by herniated calcium salts and macrophage-mediated inflammatory reaction, similar to calcific tendinitis [12–15]. Our patient exhibited muscle pain, right torticollis, and reactive thoracolumbar scoliosis. Fever in our patient was not detected, nor were sensorimotor disturbances.

Sonnabend et al. [13] demonstrated that disc herniation alone cannot explain the pain [16]. Some authors suggest that the clinical symptoms are related to the inflammation process within the disc [17, 18]. Muscle spasm could also be partly responsible for the pain. Immobilization using head halter traction or cervical collar probably limits the loading of the inflamed disc during the phase of acute inflammation and relieves the pain resulting from muscle spasm. The pain could be associated with a rise of intradiscal pressure, which partly explains the enlarged intervertebral space and adjacent decrease in vertebral body height, even though this was not the case with our patient [13, 16].

Half of the patients who underwent blood examination exhibited abnormal levels of inflammatory indicators. Erythrocyte sedimentation rate was the most sensitive indicator, elevated in more than 90% of patients. In contrast, white blood cell count and C-reactive protein showed positive reactions in only about one third of patients. We found only one study in which thyroid function tests, parathyroid hormone level assay, serum calcium, serum phosphate, serum alkaline phosphatase, peripheral blood film, and urine complete examination were done, and all the results were within normal limits [5].

For determining the presence of the condition and evaluating its progress, frontal and lateral radiographs are sufficient. CT can confirm dense calcification, show edema, and reveal an eventual herniation of *nucleus pulposus*, which can be found in up to 38% of patients, its migration into neural foramen, and consequences on the spinal cord [13, 19]. However, Ginalski et al. [20] considered in 1992 that CT is unnecessary irradiation and should only be indicated when disk calcifications are associated with neurological symptoms. MRI should be done to exclude root, spinal cord, or vertebral artery compression [16]. Symptoms such as a headache, syncope, vertigo, tinnitus, ataxia, dysarthria, visual disturbance, Horner's syndrome, vomiting, or dysphagia should alert the physician to the possibility of vertebral artery insufficiency, possibly due to herniation of disk through foramen transversarium – some authors recommend doing magnetic resonance angiography (MRA) [16]. Additional investigations (CT, MRI, and MRA) should be recommended only for patients with sensorimotor disturbances [8, 21].

When calcification is clearly visible on plain films, in the form of an oval, round shape that could be fragmented,

the diagnosis is straightforward. However, if calcification of the disc has not yet developed, the findings are more subtle, consisting primarily of bulging of the involved intervertebral disc into the adjacent vertebral bodies [11]. On MRI examinations in patients with disc calcification, there is a loss of signal on T1- and T2-weighted images. In some patients, the loss of signal in an adjacent vertebral body without the loss of vertebral height has been found [22, 23]. These changes could be the evidence that supports the concept of the vertebral body sustaining the initial insult and that disc involvement may be secondary [22]. In our study, CT scan showed sclerosation of adjacent vertebral bodies without the loss of height. This could be an indicator of the later stage of the mentioned process.

There are several hypotheses, some of which focus on the changes in the disc as the primary part of process, while others place focus on the vertebral body.

Signs of low-grade fever, leukocytosis, elevated erythrocyte sedimentation, mild pleocytosis and elevated protein in the cerebrospinal fluid support the theory of an inflammatory process during disc development or during the symptomatic interval of the disease [24]. Smith et al. [6], who performed anterior discectomy in a 12-year-old boy at the C4/C5 level, demonstrated an inflammatory response of severe reactive fibroblastic proliferation associated with multinucleated foreign body type giant cells and pleomorphic histiocytes in the disc material, while the herniated fragment of the nucleus pulposus appeared to be relatively normal. Contrary to these reports, Gerlach et al. [24] found neither evidence of inflammatory or reactive changes, nor neovascularization.

Swischuk et al. [11] focus on the disruption of blood supply to the intervertebral disk with resulting disk swelling and necrosis. In children, the discs are supplied by small blood vessels through the cartilaginous vertebral end plates. However, approximately at the age of eight, the vessels begin to obliterate. By 20 to 30 years of age, the blood vessels for the most part disappear. After this sequence of events, disc nutrition becomes nonvascular and *nucleus pulposus* starts getting nutrients by osmotic passage from the vertebral end plate. This way of transition occurs effortlessly and it appears that the process is relatively smooth. It's noted that during this transition, there is a tenuous vascular connection between the cartilaginous end plate of the vertebral body and the juxtaposed layer of the intervertebral disc. It is possible that in some patients during the critical period of nutrition transfer, some external factor could result in a premature interruption of blood/nutrition to the disc/vertebral body complex. It may be that the vertebral body becomes involved first, but in most cases escapes unharmed. It could also be that vertebral body involvement could lead to the interruption of the tenuous blood/nutrition supply from the vertebral body to the disc so the disc would become relatively ischemic, swollen, and even necrotic. Later, it would calcify. Because of swelling, the disc would bulge peripherally or into the vertebral body as a Schmorl's node. If the vertebral body is "softened" because of inflammation and vasculitis, the disc could bulge in the convex direction into the vertebral body [11].

Aforementioned mechanisms could be triggered by trauma, infection, inflammation, or vasculitis. Trauma is found in 7–30% of the cases, while respiratory infection is found in 15% of patients' histories [13]. In our case, both factors were present.

Juvenile calcification of intervertebral discs is benign and self-limiting. In most cases, conservative treatment is required. It should consist of analgesics, non-steroid anti-inflammatory drugs, muscle relaxants, cervical collar, and limitation of physical activity. Patient symptoms are relieved between five days and three weeks in two thirds of patients and in six months in 95% of patients [13, 15]. Rapid improvement in symptoms is most probably related to the natural pathophysiology of juvenile intervertebral disc calcification [16]. On radiographs, the calcification disappears within a few months, although there were cases where it persists longer [24].

Surgical treatment is rarely required. There are no guidelines for it. Nerve root or spinal cord compression by the calcified disc are not considered to be absolute indications for surgical intervention [7]. Surgical decompression should be reserved only for cases with persistent neurological deficits or progressive neurological deterioration following an inadequate course of conservative treatment [16, 24]. Anterior cervical discectomy with decompression of the spinal cord is a safe procedure with good clinical results [24].

Juvenile intervertebral disk calcification is a rare disease. For emergency medicine practitioners it's important to have this disease in mind. When found, it should be regarded as benign condition with excellent outcome by means of conservative treatment. However, the patient should be checked more frequently for the progression of calcification and neurological symptoms. If there are signs of nerve root and spinal cord compression by the calcified disk, CT scan and MRI should be performed. If neurological status deteriorates further after attempted conservative treatment, surgery should be considered.

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REFERENCES

1. Sandstrom C. Calcifications of the intervertebral discs and the relationship between various types of calcifications in the soft tissues of the body. Acta Radiol. 1951; 36(3):217–33.
2. Spapens N, Wouters C, Moens P. Thoracolumbar intervertebral disc calcifications in an 8-year-old boy: case report and review of the literature. Eur J Pediatr. 2010; 169:577–80.
3. Melnick J, Silverman F. Intervertebral disk calcification in childhood. Radiology. 1963; 80:399–408.
4. Baron A. Uber eine neue Erkrankung der Wirbelsaule. Jahrb Kinderheilkd. 1924; 104:357–60.
5. Dhammi IK, Arora A, Monga J. Calcified thoracic intervertebral disc at two levels as a cause of mid-back pain in a child: a case report. J Orthop Sci. 2002; 7(5):587–89.

6. Smith R, Vohman M, Dimon JH, Averett JE, Milsap JH. Calcified cervical intervertebral discs in children. Report of three cases. J Neurosurg. 1977; 46(2):233–8.
7. Tsutsumi S, Yasumoto Y, Ito M. Idiopathic intervertebral disk calcification in childhood: a case report and review of literature. Childs Nerv Syst. 2011; 27:1045–51.
8. Dai LY, Ye H, Qian QR. The natural history of cervical disc calcification in children. J Bone Joint Surg. 2004; 86(7):1467–72.
9. Girodias J, Azouz E, Marton D. Intervertebral disk space calcification: a report of 51 children with a review of the literature. Pediatr Radiol. 1991; 21(8):541–6.
10. Barrett M, Anderson J, Rao B, Vaslow D, Hoernschemeyer D. Multilevel pediatric cervicothoracic intervertebral disc calcifications. Am J Orthop (Belle Mead NJ). 2008; 37(7):E126–8.
11. Swischuk LE, Jubang M, Jadhav SP. Calcific discitis in children: vertebral body involvement (possible insight into etiology). Emerg Radiol. 2008; 15(6):427–30.
12. Coventry MB. Calcification in a cervical disc with anterior protrusion and dysphagia. J Bone Joint Surg Am. 1970; 52(7):1463–6.
13. Sonnabend DH, Taylor TK, Chapman GK. Intervertebral disc calcification syndromes in children. J Bone Joint Surg Br. 1982; 64(1):25–31.
14. Jawish R, Ponet M. Dysphagia disclosing a cervical disk calcification in a child:apropos of a case and review of the literature. Chir Pediatr. 1990; 31(2):127–30.
15. Kim H, Ha D, Lee S, Kim S, Wenokor C. Acute Symptomatic Intervertebral Disk Calcification in a Child With Retropharyngeal Edema. J Clin Rheumatol. 2011; 17(7):365–7.
16. Mac-Thiong JM, Leduc S, Marton D, Duhaime M, Morin B. Herniation of a Calcified Cervical Disc into the Foramen Transversarium in an 8-Year-Old Child. Spine. 2004; 29(16):349–52.
17. Pattekar MA, Krishnan A, Kazmierczak C. Juvenile intervertebral disk calcification: a cause of painful torticollis. Emerg Radiol. 2001; 8(6):338–40.
18. Bagatur AE, Zorer G, Centel T. Natural history of paediatric intervertebral disc calcification. Arch Orthop Trauma Surg. 2001; 121(10):601–3.
19. Lernout C, Haas H, Rubio A, Griffet J. Pediatric intervertebral disk calcification in childhood: three case reports and review of literature. Childs Nerv Syst. 2009; 25(8):1019–23.
20. Ginalski JM, Landry M, Gudinchet F, Schnyder P. Is tomography of intervertebral disc calcification useful in children? Pediatr Radiol. 1992; 22:9–61.
21. Bajard X, Renault F, Benharrats T, Mary P, Madi F, Vialle R. Intervertebral disc calcification with neurological symptoms in children: report of conservative treatment in two cases. Childs Nerv Syst. 2010; 26(7):973–8.
22. Swischuk L, Stansberry S. Calcific discitis: MRI changes in disks without visible calcification. Pediatr Radiol. 1991; 21:365–6.
23. Herring JA, Hensinger RN. Instructional case cervical disc calcification. J Pediatr Orthop. 1988; 8:613–6.
24. Gerlach R, Zimmermann M, Kellermann S, Lietz R, Raabe A, Seifert V. Intervertebral disc calcification in childhood--a case report and review of the literature. Acta Neurochir Wien. 2001; 143(1):89–93.

Калцификација међупршљенског диска вратне кичме код детета – приказ случаја и преглед литературе

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САЖЕТАК

Увод Представљамо случај калцификације интервертебралног диска вратне кичме код детета. Ово ретко обољење је описано у око 400 случајева широм света. Оболела деца долазе код лекара са тегобама у виду болова у врату, мишићним спазмом и рендгенским снимком калцификације интервертебралног диска. Наш циљ био је да прикажемо случај изненадне појаве бола у врату и тортиколиса.

Приказ болесника Болесница се јавила после трауме, са боловима у врату и спазмом десног стерноклеидомастоидног мишића. Иницијално је направљен рендгенски снимак, када је откривена калцификација испред *C4* и *C5* вратног кичменог пршљена, а затим је начињен *CT* снимак вратне кичме. Када је искључено постојање компресије коренова спиналних живаца, укључен је конзервативни третман. После две недеље дете је било без тегоба.

Закључак Иако постојање сенке са предње стране кичменог стуба изазива забринутост, доктори ургентне медицине треба да имају у виду да је ово обољење у већини случајева бенигне природе, те одлично реагује на конзервативно лечење.

Кључне речи: калцификација; интервертебрални диск; вратни бол; тортиколис

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Hysteroscopy – history and development

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SUMMARY
Hysteroscopy is the gold standard for diagnosing and managing endocervical and endometrial pathology. The development of today's hysteroscopy begins in the early 19th century. Initially, hysteroscopy was used solely for diagnostics. Operative hysteroscopy surfaced with the development of distension media, the hysteroscope, and its associated instruments. Operative hysteroscopy underwent the most significant development in the early 1970s, when new hysteroscopes were introduced, and the distension media became more widely used. A multitude of hysteroscopic procedures are performed with the common goal of removing pathological changes in the endometrial cavity.
In the 1980s, small cameras, also known as "chip" cameras, were developed, leading to the transition of endoscopy into videoendoscopy. Bettocchi revolutionized modern hysteroscopy in 1996 when he used the first operative office hysteroscope. Operative resectoscopes, containing monopolar and bipolar energy, were also constructed. Hysteroscopic morcellators have been in use since the beginning of the 21st century. Today's modern hysteroscopy represents a safe diagnostic and operative endoscopy.
Keywords: hysteroscope; resectoscope; morcellator; operative hysteroscopy

INTRODUCTION

Hysteroscopy is an endoscopic procedure, which can diagnose and treat the pathology of the endometrium, tubal ostia, and cervical canal. Hysteroscopy can be diagnostic and/or operative. The name "hysteroscopy" originates from Greek words *ύστέρα* – uterus and *σκοπέω* – to look. Hysteroscopy is the oldest endoscopic method in gynecologic surgery. The theoretical concept of hysteroscopy dates back to BC Babylon of Mesopotamia. An instrument, similar to today's speculum, was discovered on Babylonian plaques, which was used to determine whether bleeding was of uterine or vaginal origin [1, 2].

Modern hysteroscopy has been developing since the beginning of the 19th century. New optic hysteroscopes were built, and carbon dioxide was used as a distention medium for the uterine cavity. In the 1960s, hysteroscopes were constructed with a one-way canal for irrigation. At the beginning of the 1970s, operative hysteroscopy started developing. The construction of a two-way distention medium delivery system, modern instruments, monopolar and bipolar energy generators, hysteroscopic pumps, resectoscopes and fluid distention media contributed to the development of modern hysteroscopy in the 1980s. The development of an endocamera enabled the use of video hysteroscopy. Operative office hysteroscopes were developed at the end of the 20th century. Hysteroscopic morcellators were developed at the beginning of the 21st century. These instruments shorten the operative time and decrease risk of complications. The ongoing technological advancement enables innovation of instruments and equipment, which advance operative technique leading to diagnosis and management of pathological intrauterine changes [3, 4].

THE DEVELOPMENT OF HYSTEROSCOPY IN THE 19TH CENTURY

Modern hysteroscopy originates from Philip Bozzini's articles in 1807 where he described the endometrial cavity, the existence of intrauterine tumours and malformations**.** He used a candle and a concave mirror as an illumination method (Figure 1). In 1853, Desormeaux developed the first cystoscope, and it was used to perform the first cystoscopy [2]. The cystoscope had two channels, one for introduction of liquids and the other for instruments. He used

Figure 1. Bozzini's Lichtleiter or light conductor

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Figure 2. Desormeaux's endoscope

a lamp and a concave mirror as an illumination method (Figure 2).

The first hysteroscopy on an actual patient was performed in 1869 by Pantaleoni. Pantaleoni used the cystoscope developed by Desormeaux. He discovered that an endometrial polyp was the source of bleeding in a 60-year-old woman, and treated it with silver nitrate. A second generation of cystoscope was constructed in 1879 by Nitze, which contained optic lenses. In 1898, Duplay and Clado published the first book on hysteroscopy [4].

THE DEVELOPMENT OF HYSTEROSCOPY IN THE FIRST HALF OF THE 20TH CENTURY

In 1907, David constructed the first contact hysteroscope, and performed the first optic hysteroscopy. However, the endometrial visualization was not optimal, as contact hysteroscopy did not use a distension medium. In order to improve the visualization of the endometrial cavity, it was necessary to distend the endometrial cavity during optic observation. For this reason, Heinberg constructed an irrigation system in 1914. In 1925, Rubin constructed a hysteroscope and carbon dioxide was used as the distension medium [2]. Seymour constructed a hysteroscope for introduction and suction of the distension medium in 1926. The first hysteroscope with an operative channel was developed by Mikulicz-Radecki and Freund in 1927, which enabled endometrial biopsy by ocular observation. The hysteroscope equipped with an irrigation system was built by Gauss in 1928 [5]. In 1934, Schroeder improved the hysteroscope by placing the lens at the top, which also improved visualization. A liquid with properties similar to water was used as the distension medium. He used the hysteroscope to diagnose endometrial polyps, submucous fibroids and to determine the menstrual cycle phase [2]. In 1936, Second designed the optic hysteroscope with a fluid delivery system and fixed optics. Palmer directly visualized the endocervical canal during hysteroscopy in 1942, and suggested this be the standard of care during all hysteroscopic procedures. Norment placed the light source on the proximal end of the hysteroscope and by doing so, contact hysteroscopes were modified in 1947 [5].

DEVELOPMENT OF HYSTEROSCOPY IN THE SECOND HALF OF THE 20TH CENTURY

The advancement of hysteroscopy during this period was marked by the introduction of new distension media and operative hysteroscopy (i.e. performing operative procedures using the hysteroscope). In 1962, Silander used a hysteroscope with two channels, an internal canal for observation and an external for irrigation. The distal end of the hysteroscope had a lamp and thin balloon. By alternating the pressure in the balloon and maneuvering the hysteroscope, it was possible to examine the endometrial cavity in great detail [2]. A fiberoptic cable was built into the hysteroscope in 1965. The illumination was accomplished using cold xenon light, and the light source was located outside the body.

The development of modern hysteroscopy began in the 1970s. From 1980, hysteroscopy was accepted as the standard procedure to diagnose and treat endometrial cavity and endocervical canal pathology [6]. New hysteroscopic instruments were developed, and the distension media became more widely used. Office hysteroscopy was performed in outpatient setting without the use of an anesthetic. Operative hysteroscopic procedures continued to develop and became more widely utilized [7].

The available types of hysteroscopes included rigid, flexible, and contact. Hysteroscopes are available in different diameters with different observation angles [2]. The most commonly used is the rigid hysteroscope, which consists of a telescope with a diameter of 2.9 mm, a 30° angle, and an outer sheath of 5 mm (Figure 3). The first flexible hysteroscope was constructed by Mohri in 1971. These hysteroscopes are fiberoptic, ranging from 3.1 mm to 3.7 mm, and are used for diagnostics. Semi-rigid fiberoptic mini hysteroscopes Verascope (Gynecare, Ethicon, Somerville, NJ, USA) have a 1.8 mm telescope, 0° angle and an outer sheath of 3.2 mm. These hysteroscopes are used for diagnostic and operative procedures. These hysteroscope can use mechanical instruments, such as scissors, grasper and biopsy forceps, as well as 5Fr bipolar electrodes for resection, coagulation, and vaporization.

Since 1970, the use of new media for the distension of the uterine cavity begins. Edstrom (1970) used 32% dextrose (MW 70 000 Daltons) as the distension medium during biopsy of endometrial lesions. Since then, a few media have been used successfully to distend the endometrial cavity [8]. Lindemann (1971) used carbon dioxide as the distension medium. Menken (1972) used the high-molecular weight solution polyvinylpyrrolidone as the distension medium, while Sugimoto (1975) used isotonic

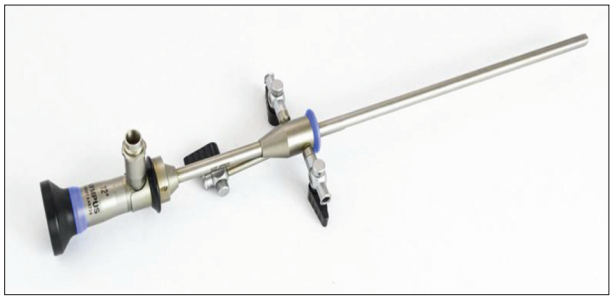

Figure 3. Rigid hysteroscope

Figure 4. Bettocchi office operative hysteroscope

Figure 5. Olympus bipolar resectoscope

sodium chloride and Quinones-Guerrero (1976) used 5% dextrose [9, 10].

Hysteroscopic procedures became possible, and have continuously improved with the use of distension media and development of new instruments. Female sterilization was performed using operative hysteroscopy by Menken in 1971. Lindemann and Quinones-Guerrero successfully performed transcervical catheterization of fallopian tubes using hysteroscopy in 1972. The treatment of intrauterine adhesions using hysteroscopy was first performed by Porto in 1973. In 1974, Edstrom used the biopsy forceps for intrauterine adhesiolysis, as well as to perform metroplasty i.e. resection of uterine septum. In 1976, the first hysteroscopic transcervical resection of a submucous fibroid using an urologic resectoscope, monopolar current and 32% dextran 70 as distension medium was done by Neuwirth and Amin [2, 11]. A resectoscope was used for hysteroscopic myomectomy by Hanning and colleagues in 1980. Hysteroscopic laser endometrial ablation was performed by Goldrath and colleagues in 1981 [12].

The charge-coupled device (CCD), also known as the "chip," was developed in 1982 consisting of a small endocamera and a sensor, which led to the transition from endoscopy to videoendoscopy**.** The high definition (HD) camera was developed later**.** De Cherny and colleagues used a resectoscope in 1986 to resect a uterine septum. Hysteroscopic myomectomy using a Nd:YAG laser was performed by Baggish in 1989. Hysteroscopic resection of a uterine septum with a Nd:YAG laser was performed by Choe in 1992. Fedele and colleagues used an argon laser to resect a uterine septum in 1993 [13, 14].

In 1996, Bettocchi developed the first "office" operative hysteroscope, which was used for outpatient procedures. It consisted of a telescope of 2.9 mm diameter, covered with a continuous flow sheath and an operating sheath equipped with a channel for semi-rigid 5Fr surgical instruments and electrodes (Figure 4). The hysteroscope can be inserted into the uterus without cervical dilatation, and it enables the operative procedure to be performed in outpatient settings. The Bettocchi office hysteroscope with telescope of 2 mm diameter, simple flow, and outer 3.6 mm sheath is used for diagnostic hysteroscopy. There is the option of using the Bettocci Integrated Office Hysteroscope (B.I.O.H.) diameter 4mm, for diagnostic and office-based procedures [15, 16, 17].

Further development of technology led to the construction of the operative resectoscope, which uses monopolar energy. The resectoscope is used for operative procedures, and consists of a telescope ranging 2–4 mm, 12º angle, two sheaths with irrigation and suction channels, and an operative part that bears the cut/coagulation electrodes. The resectoscope is connected to a high frequency electrosurgical unit. When using high frequency monopolar energy for operative hysteroscopy, the distension medium must be without electrolytes (i.e. 4% sorbitol-mannitol, 1.5% glycine, 5% glucose). After that, a bipolar resectoscopes have been constructed by several manufacturers, including Karl Storz (Tuttlingen, Germany) with 22 and 26Fr diameters, as well as Olympus Corporation (Tokyo, Japan) bipolar resectoscope has an 8.5 mm outer sheath, with a 12º telescope (Figure 5) and Richard Wolf Medical Instruments Corporation (Vernon Hill, IL, USA) princess resectoscope with 21Fr diameter. Bipolar resectoscopes enable transcervical submucous fibroid resection, uterine septum resection, endometrial polyp removal, endometrial biopsy and ablation, and intrauterine adhesiolysis [18, 19]. Utilization of this resectoscope enables the use of distension media that have a low viscosity and contain electrolytes, such as 0.9% sodium chloride and Ringer's lactate, which reduces the risk of hyponatremia and transurethral resection of the prostate syndrome during the surgical procedure [20].

The advancement of monopolar and bipolar electrosurgical technology enabled the introduction and utilization of multipurpose electrosurgical systems. This includes the Gynecare Versapoint bipolar electrosurgery system, which has been in use since 1997. This system contains high-frequency electrosurgical generator and a bipolar resectoscope. Modern high-frequency electrosurgical unit Autocon 400 (Karl Storz) can be operated both in unipolar and bipolar mode [21]. An intelligent electrosurgical unit was also constructed, the Olympus UES-40 SurgMaster

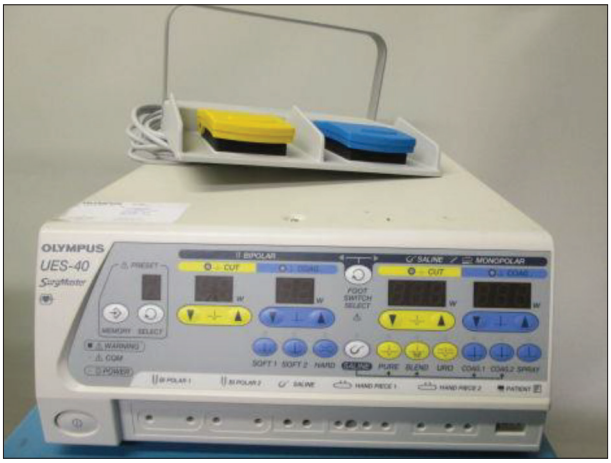

Figure 6. Olympus UES-40 SurgMaster electrosurgical unit

Figure 7. TRUCLEAR hysteroscopic morcellator

generator, as a source of monopolar and bipolar energy, which enables the utilization of the bipolar resectoscope in normal saline (Figure 6).

THE DEVELOPMENT OF HYSTEROSCOPY AT THE BEGINNING OF THE 21ST CENTURY

The beginning of the 21st century is marked by increasing advancement and usage of operative hysteroscopy. With the usage of bipolar energy, laser energy and various instruments, hysteroscopy is a safe method to perform numerous surgical procedures due to the low incidence of complications [22, 23]. Intrauterine morcellation is a newer approach to operative hysteroscopy that removes uterine pathology under direct visualization with continuous real-time tissue fragment removal. Hysteroscopic morcellators were constructed to decrease the operative time and increase the procedural safety. In 2005, the American Food and Drug Administration (FDA), approved the use of the first hysteroscopic morcellator "TRUCLEAR" (Smith and Nephew, Andover, MA, USA) (Figure 7). The morcellator is 9 mm in diameter, and has a rotating blade, which enables the removal of fibroid and polyp tissue from the endometrial cavity, which can then be sent for histopathological examination [24, 25]. The FDA also approved the use of "MyoSure tissue removal system" (Hologic, Bedford, MA, USA) in 2009, which is another type of morcellator, measuring 6.25 mm in diameter with a rotating and oscillating blade. This morcellator can be used to remove submucous fibroids, which measure up to 3 cm in diameter. A mechanical hysteroscopic morcellator is also available and includes the "Bigatti shaver" (Karl Storz) [26, 27, 28].

CONCLUSION

Hysteroscopy represents the gold standard for diagnosis and treatment of endocervical and endometrial cavity pathology. It is a modern and safe endoscopic method, which enables numerous surgical procedures, such as endometrial biopsy, endometrial polyp removal, submucous fibroid resection, uterine septum resection, tubal catheterization, and hysteroscopic sterilization.

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REFERENCES

1. Russell JB. History and development of hysteroscopy. Obstet Gynecol Clin North Am. 1988; 15(1):1–11.
2. Magos A. History and evolution of hysteroscopy. In: Shawki O, Deshmukh S, Pacheco LA, editors. Mastering the techniques in hysteroscopy. New Delhi, London, Panama: Jaype Brothers Medical Publishers; 2017. p. 8–21.
3. Centini G, Troia L, Lazzeri L, Petraglia F, Luisi S. Modern operative hysteroscopy. Minerva Ginecol. 2016; 68(2):126–32.
4. Blanc B, Marty R, Montgolfier R. Office and operative hysteroscopy. Paris: Werlag Springer; 2013. (e-Book).
5. Sugimoto O. A color atlas of hysteroscopy. Tokyo: Springer-Verlag; 1999.
6. Indman PD. Instrumentation and distension media for the hysteroscopic tretament of abnormal uterine bleeding. Obstet Gynecol Clin North Am. 2000; 27(2):305–15.
7. Valle RF. Development of hysteroscopy from a dream to a reality and its linkage to the present and future. J Minim Inv Gynecol. 2007; 14(4):407–18.
8. El Huseiny AM, Soliman BS. Hysteroscopic findings in infertile women: a retrospective study. Middle East Fertility Society Journal. 2013; 18(3):154–8.
9. Neuwirth RS. Hysteroscopy and gynecology: past, present and future. JMIG. 2001; 8(2):193–8.
10. Batra N, Khnda A, Donovan P. Hysteroscopic myomectomy. Obstet Gynecol Clin North Am. 2004; 31:669–85.
11. Mazzon I, Favilli A, Cocco P, Crasso M, Horvath S, Bini V, et al. Does cold loop hysteroscopic myomectomy reduce intrauterine adhesions? A retrospective study. Fertil Steril. 2014; 101(1):294–8e3.
12. Perino A, Castelli A, Cucinella G, Biondo A, Pane A, Venezia R. A randomized comparison of endometrial laser intrauterine thermotherapy and hysteroscopic endometrial resection. Fertil Steril. 2004; 82(3):731–4.
13. Rock JA, Jones III HW. Te Lindes operative gynecology. 11th ed. Philadelphia: Lippincot Williams & Wilkins; 2015.

14. Esmaeilzadeh S, Delavar MA, Andarieh MG. Reproductive outcome following hysteroscopic treatment of uterine septum. Mater Sociomed. 2014; 26(2):366–71.
15. Bettocchi S, Ceci O, Nappi L, Di Venere R, Masciopinto V, Pansini V. Operative office hysteroscopy without anesthesia: analysis of 4863 cases performed with mechanical instruments. J Am Assoc Gynecol Laparosc. 2004; 11(1):59–61.
16. Raimondo G, Raimondo D, Aniello G, Russo C, Ronga A, Gabbanini M, et al. A randomized controlled study comparing carbon dioxide versus normal saline as distension media in diagnostic office hysteroscopy: is the distension with carbon dioxide a problem? Fertil Steril. 2010; 94(6):2319–22.
17. Bettocchi S, Achilarre MT, Ceci O, Luigi S. Fertility-enhancing hysteroscopic surgery. Semin Reprod Med. 2011; 29(2):75–82.
18. Di Spiezio Sardo A, Calagna G, Di Carlo C, Guida M, Perino A, Nappi C. Cold loops applied to bipolar resectoscope: A safe „one-step" myomectomy for treatment of submucosal myomas with intramural development. J Obstet Gynaecol Res. 2015; 41(12):1935–41.
19. Casadio P, Guasina F, Morra C, Talamo MT, Leggieri C, Frisoni J, et al. Hysteroscopic myomectomy: techniques and preoperative assessment. Minerva Ginecol. 2016; 68(2):154–66.
20. Johansson J, Lindahl M, Gyllencreutz E, Hahn RG. Symptomatic absorption of isotonic saline during transcervical endometrial resection. Acta Anaesthesiol Scand. 2017; 61(1):121–4.
21. Mencanglia L, Cavalcanti L, Neto A, Alvarez AA. Manual of hysteroscopy-diagnostic, operative and office hysteroscopy. Tuttingen: Endo Press; 2013.
22. Munro MG, Christianson LA. Complications of hysteroscopic and uterine resectoscopic surgery. Clin Obstet Gynecol. 2015; 58(4):765–97.
23. Nappi L, Sorrentino F, Angioni S, Pontis A, Litta P, Greco P. Feasibility of hysteroscopic endometrial polypectomy using a new dual wavelengths laser system (DWLS): preliminary results of a pilot study. Arch Gynecol Obstet. 2017; 295(1):3–7.
24. Emanuel MH, Wamsteker K. The intra uterine morcellator: a new hysteroscopic operating technique to remove intrauterine polyps and myomas. J Minim Invasive Gynecol. *2005; 12(1):62–6.*
25. Li C, Dai Z, Gong Y, Xie B, Wang B. A systematic review and meta-analysis of randomized controlled trials comparing hysteroscopic morcellation with resectoscopy for patients with endometrial lesions. Int J Gynaecol Obstet. 2017; 136(1):6–12.
26. Cohen S, Greenburg JA. Hysteroscopic morcellation for treating intrauterine pathology. Rev Obstet Gynaecol. 2011; 4(2):73–80.
27. Lee M, Matsuzono T. Hysteroscopic intrauterine morselation of submucosal fibroids: preliminary results in Hong Kong and comparisons with conventional hysteroscopic monopolar loop resection. Hong Kong Med J. 2016; 22(1):56–61.
28. Bigatti G, Ferrario C, Rosales M, Baglioni A, Bianchi S. A 4-cm G2 cervical submucosal myoma removed with the IBS^{R} Integrated Bigatti Shaver. Gynecol Surg. 2012; 9(4):453–6.

Хистероскопија – историја и развој

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САДРЖАЈ

Хистероскопија представља златни стандард за дијагнозу и третман ендоцервикалне патологије и патологије шупљине материце. Развој данашње хистероскопије почиње почетком XIX века. У почетку се хистероскопија користила у дијагностичке сврхе. Увођењем у употребу медијума за дистензију материчне шупљине и конструкцијом хистероскопа и инструмената почиње да се развија и оперативна хистероскопија. Најзначајнији развој оперативне хистероскопије почиње седамдесетих година XX века. Конструисани су и нови хистероскопи и почиње све шира употреба медијума за дистензију материчне шупљине. Хистероскопским путем се изводе бројне хируршке процедуре у циљу уклањања патолошких промена у шупљини материце.

Осамдесетих година XX века конструисане су и мале ендокамере, чип камере, па тако ендоскопија прераста у видеоендоскопију. Револуцију у свету модерне хистероскопије представља 1996. година када је Беточи пројектовао и употребио први оперативни *office* хистероскоп. Конструисани су и оперативни ресектоскопи, који користе монополарну и биполарну струју. Почетком XXI века конструисани су и почињу да се примењују и хистероскопски морселатори. Данашња модерна хистероскопија представља безбедну дијагностичку и оперативну ендоскопску процедуру.

Кључне речи: хистероскоп; ресектоскоп; морселатор; оперативна хистероскопија

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REGULATORY STANDARDS IN MEDICINE / РЕГУЛАТОРНИ СТАНДАРДИ У МЕДИЦИЈИ

Criminal responsibility for medical malpractice in the jurisdictional practice of Serbia

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SUMMARY

In the field of protection and improvement of people's health, there is a special importance of legally, efficiently, regularly, professionally, and punctually providing medical care, performing other healthcare services, or simply providing medical assistance or care. In this way, an essential social function is achieved, as well as the protection of the constitutionally proclaimed right of physical and mental integrity of the public. However, deterioration of an individual's health who has been medically assisted is possible in the process of providing medical, or any other assistance in the field of medicine.

If it is a gross medical misconduct or any other type of medical misconduct, or gross violation of a profession's rules, because of which there is a possibility of deterioration of health of one or more individuals, then the crime of medical negligence, for which there are strict statutory offences, applies. This article addresses the aspect of theory and practice about the significance, social jeopardy, and prevalence of this crime, or criminal policy of courts in the Republic of Serbia, alongside many articles in the printed and electronic media which provoke great public attention and rough comments.

Keywords: health; crime; responsibility; criminal sanction; policy of the criminal prosecution; court crime policies

INTRODUCTION

People's healthcare, along with life protection and bodily integrity, represents a social function which every country has performed from the early ages to the present. This is proven by many crimes from which these personal and social values are protected. However, the care has not always been complete, efficient, evenly distributed, and general. There had been minor or greater differences in incriminations of violating or imperiling these social values, depending on the characteristics and the type of a state organization. Since the bourgeois revolution in France, the protection of these social values has gained significance, considering the declared human rights and freedoms. The protection of these human rights was proclaimed as a part of universal (United Nations) and regional (Council of Europe) international documents and constitutions of states as the highest legal acts.

All positive criminal legislations regulate various forms and aspects of manifestations of the crimes against health. The situation is similar in Serbia, where a Criminal Code has been in effect since January 1, 2006 in which in Chapter 23, titled Offences Against Human Health, more felonies against people's welfare are anticipated [1]. Namely, these are crimes against not only the people's wellbeing, but also against the right to protect one's health, guaranteed in Article 68 of the Constitution of the Republic of Serbia.

Among the crimes against human welfare there is a crime which by its significance, nature, characteristics, perpetrator, the type and scope of caused consequences is singled out from Article 251 of the Criminal Code, titled Medical Malpractice. In the protection of human health, medical assistance or care for the ill has special significance. People who are authorized to provide this kind of assistance – medical doctors or other health workers – apart from the appropriate professional education should act responsibly and in accordance with the regulations of the medical profession, science, and skill. Hence, there is the need for stronger criminal relief for the ill. Moreover, in legal theory there are positions that this is the case of professional crime, or the crime of professional negligence.

The crime from Article 251 consists of irresponsible medical assistance provided by a doctor, or irresponsible medical assistance, care, or other medical service provided by any other healthcare practitioner, which results in health deterioration of an individual. If the perpetrator of the crime proves to be a doctor or other medical practitioner, they will be imprisoned between three months and three years. Unpremeditated act, on the other hand, results in a fine or in imprisonment of up to one year.

In order to recognize the position of the crime of medical malpractice in relation to the modern Serbian law, it is necessary to look into the analysis of Serbia's jurisdictional practice. Accordingly, legal regulations are not only abstract terms in legal acts, but also exist in the everyday police, jurisdiction, and prosecution practice in various forms and aspects. In this

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analysis, we used available data, previously published in reports of the Statistical Office of the Republic of Serbia in the 2006–2015 period, which is the exact period of applying current positive criminal legislations [2–11].

THE PATTERN AND EXTENT OF OFFENCES AGAINST HUMAN HEALTH

To begin with, we are going to analyze the state of crime in general and then the state of offences against human health, as crimes in Serbia in general in the 2006–2015 period. Afterwards, we are going to analyze the extent, pattern, and tendencies of manifestations of medical malpractice (Table 1).

From the collected data on crime in general and the crimes against health, we can conclude the following:

1) The highest number of crimes in Serbia was 108,750, in the lastly analyzed year of 2015, while the lowest number of crimes was 74,279 in 2010; since then, the number of crimes has been rising steadily;

2) In contrast, the highest number of crimes against health was 4,895 in 2008, while the lowest number was only 3,161, in 2014;

3) Even though it is considered that crimes against health are significantly involved in the total number of crimes in the state (especially the crimes against people's health that involve drugs and psychoactive substances), this cannot be concluded from the collected data. Namely, health crimes are a part of crimes in general, with 5.46% in 2010 (the period with the greatest share), 4.81% in 2008, with the smallest share of these crimes in total number of crimes, and 3.43% in 2015, or 3.41% in 2014 (Table 2).

After the opening statements about the crime rate in general and the rate of crime against health in Serbia in the 2006–2015 period, we shall analyze the pattern and the extent of medical malpractice manifestations from Article 251 of the Criminal Code. From the mentioned analysis on the crime rate, we can conclude the following: 1) the highest number of medical malpractice offences, committed in 2015, was 101 (2.71%); 87 (2.75%) were committed in 2014 (these were the years when the highest percentage share in offences against health was noted); 2) observed number of crimes committed in 2011 was only 39, the percentage was 1.02% in 2009, and 1.08% in 2008.

CRIMINAL PROSECUTION POLICY

The statements about the pattern, extent, structure, and tendencies manifested by offences against human health in Serbia in general or medical malpractice need to be followed by statements about the criminal prosecution policy for perpetrators of such crimes. Namely, the total number of reported crimes, which has been shown previously, is not the real number of committed crimes in general, as well as the crimes against health, since there has always been a "gray" or "dark" number of crimes or the loss of crimes.

Table 1. Medical malpractice involvement in the total number of crimes in Serbia in the 2006–2015 period

Year	Total number of crimes	Crimes against health	%
2006	105,701	4,260	4.03
2007	98,702	4,440	4.5
2008	101,723	4,895	4.81
2009	100,026	4,490	4.49
2010	74,279	4,052	5.46
2011	88,207	3,409	3.86
2012	92,879	3,603	3.88
2013	91,411	3,464	3.79
2014	92,600	3,161	3.41
2015	108,759	3,731	3.43

Table 2. Medical negligence involvement (Article 251, Paragraph 3) in offences against human health in Serbia in the 2006–2015 period

Year	Crimes against health	Crimes from Article 251 Paragraph 3	%
2006	4,260	68	1.6
2007	4,440	85	1.91
2008	4,895	53	1.08
2009	4,490	46	1.02
2010	4,052	47	1.16
2011	3,409	39	1.14
2012	3,603	58	1.61
2013	3,464	76	2.19
2014	3,161	87	2.75
2015	3,731	101	2.71

However, the data about the ratio between the reported and accused individuals for crimes in general, or for specific crimes, is quite interesting. Accordingly, from the total number of reported medical malpractice crimes, the number of the accused is significantly lower, which is legally supported (rejecting the appeal, adjournment or discontinuance of proceedings); hence, courts of competent jurisdiction accept significantly lower number of individuals in comparison to the number of the reported ones, or their crimes (Table 3).

In order for a statement on medical malpractice to be comprehensive and complete in the modern criminal law, it is necessary to take a closer look at the place, the time, and its analysis in the statistical practice of Serbia, apart from the theoretical analysis of the positive legal solutions for these crimes, or its forms and types of manifestations in the legal theory and court practice.

From the collected statistical data on criminal prosecution of perpetrators of this crime, we can conclude the following:

1) There was no interruption of investigation at any time for this crime;

2) The number of terminations of this crime is slightly higher because of the law. Hence, investigation was mostly terminated in 2007, in 9.41% of the cases; in 2006 investigation was terminated in 8.82% of the cases (thus, almost every 10th report was dismissed by terminating the investigation);

3) When it comes to this particular crime, there is a great number of rejected reports. The highest number of

Table 3. The way of termination of the previous proceeding for a medical malpractice crime in Serbia in the 2006–2015 period

Year	Number of reports	Rejected reports	%	Interrupted proceeding	%	Terminated proceeding	%
2006	68	31	45.59	0	0	6	8.82
2007	85	46	54.12	0	0	8	9.41
2008	53	28	52.83	0	0	2	3.77
2009	46	36	78.26	0	0	0	0
2010	47	28	59.57	0	0	4	8.51
2011	39	26	66.66	0	0	1	2.56
2012	58	20	34.48	0	0	5	8.62
2013	76	41	53.95	0	0	0	0
2014	87	61	70.11	0	0	1	1.15
2015	101	89	88.12	0	0	0	0

rejected reports was in 2015, with 89 out of 101 reports declined (88.12%). Usually, the number of rejected reports was more than one half of the submitted reports, except in 2012, when "only" 24.47% of the reports was rejected, and in 2006, when 45.59% of the reports was rejected.

THE CRIMINAL POLICY OF COURTS

In order to completely examine the efficiency of measures and agents which serve the state or the society, for facing and terminating various forms and types of manifestations of modern crime in general, as well as crime against health, we have to look further at the place, time, and the analysis of the criminal policy of courts. Reportedly, it is the analysis and comparison of the number of reported, accused, and sentenced individuals for the crime of medical malpractice which represents the object of our empirical examination in Serbia in the period of 2006–2015, or the analysis of the type and gravity of punishments, as well as other criminal sanctions for perpetrators of this crime.

Crimes against health are the type of crime for which all modern legislations (including the legislation of Serbia) impose sentences of imprisonment for different periods of time. However, although imprisonment is the punishment for the crime of medical malpractice, in most of the cases, other criminal sanctions are also imposed. This shows the final result of the state's consistency of confronting these crimes and the efficiency of applied sanctions set by courts in the criminal procedure (Table 4).

Table 4. The ratio of individuals reported, accused of, and sentenced for medical malpractice in Serbia in the 2006–2015 period

Year	Reported	Accused	%	Sentenced	%
2006	68	13	19.12	2	2.94
2007	85	27	31.76	8	9.41
2008	53	28	52.83	5	9.43
2009	46	22	47.83	7	15.22
2010	47	14	29.79	3	6.38
2011	39	14	35.90	6	15.38
2012	58	22	37.93	9	15.52
2013	76	43	56.58	7	9.21
2014	87	29	33.33	5	5.75
2015	101	15	14.85	3	2.97

Upon analyzing the data on the ratio between reported, accused, and sentenced individuals for the crime of medical malpractice in the observed period of time, we can conclude the following:

1) The percentage of the accused in comparison to the reported varies;

2) The percentage of the accused out of the total number of the reported individuals was the lowest in 2015 (19.85%), and in in 2006 (19.12%);

3) The greatest percentage of accused individuals was in 2013 and it amounted to 56.58%;

4) When it comes to the number of the accused in comparison to the number of the reported, the situation is not very good. This is supported by the fact that the greatest percentage of the accused (every sixth of the reported), was 15.38% in 2011, and 15.22% in 2012 and 2007;

5) Exceptionally small share of the sentenced for this particular crime, 2.94%, was in 2006; the percentage was 2.97% in 2015 (Table 5).

So that we could better understand the difference between the accused and the reported individuals for the analysis of the crime of medical malpractice, we shall analyze the ways of solving the reported crimes. From the collected data we can conclude the following:

1) The percentage of terminated procedure cases varies from the minimum of 0.99% in 2015 and 1.72% in 2012 to the maximum of 13.21% in 2008 and 10.59% in 2008;

2) The percentage of individuals who are legally exculpated varies in accordance with the year of the analysis; the minimum was 2.13% in 2010, while the percentage amounted to 19.56% in 2009, and to 18.87% in 2008;

3) It is similar with individuals for whom the report was rejected; the percentage varies from the minimum of 0.99% in 2015 or 2.56% in 2011 to the maximum of 13.16% in 2013.

It is necessary to analyze the severity or lenience of the courts' punishment policies for this crime. This can be done by observing the type of imprisonment sentences (considering the legally imposed punishment for the crime), as well as other types of punishment (Table 6).

We are going to analyze imposed penalties for medical malpractice because this punishment is the only one resolved in the Criminal Code. However, the collected data shows that even this statutory punishment was rarely imposed on the perpetrators of the crimes against health.

Table 5. Ways of solving a reported medical malpractice crime in the Serbia in the 2006–2015 period

Year	Proclaimed guilty	%	Terminated procedure	%	Exculpated	%	Accusation dismissed	%
2006	2	2.94	3	4.41	2	2.94	3	4.41
2007	8	9.41	9	10.59	3	3.53	6	7.06
2008	5	9.43	7	13.21	10	18.87	4	7.55
2009	7	15.22	2	4.34	9	19.56	2	4.34
2010	3	6.38	4	8.51	1	2.13	2	4.26
2011	6	15.38	4	10.26	3	7.69	1	2.56
2012	9	15.52	1	1.72	10	17.24	2	3.45
2013	7	9.21	6	7.89	9	11.84	10	13.16
2014	5	5.75	7	8.05	7	8.05	7	8.05
2015	3	2.97	1	0.99	10	9.9	1	0.99

Table 6. Imposed imprisonment penalties for perpetrators of the crime of medical malpractice in Serbia in the 20062015 period

Year	Sentenced	Imprisonment of 2–3 years	Imprisonment of 1–2 years	Imprisonment of 6 months to 1 year	Imprisonment of 3–6 months	Imprisonment of 2–3 months
2006	2	0	0	0	0	0
2007	8	0	0	2	1	0
2008	5	0	0	0	0	0
2009	7	0	0	0	2	0
2010	3	0	0	0	0	0
2011	6	0	0	0	1	0
2012	9	1	0	1	1	0
2013	7	0	0	1	0	0
2014	5	0	0	0	2	0
2015	3	1	0	0	0	0

Table 7. Other types of imposed sanctions for perpetrators of medical malpractice in Serbia in the 2006–2015 period

Year	Sentenced individuals	Fine payment	Probation	Warning by court	Corrective measures	Exculpated	Additional fine
2006	2	1	1	0	0	0	0
2007	8	0	5	0	0	0	0
2008	5	0	4	0	0	0	0
2009	7	0	5	0	0	0	0
2010	3	0	3	0	0	0	0
2011	6	0	5	0	0	0	0
2012	9	3	2	0	0	0	0
2013	7	0	6	0	0	0	0
2014	5	0	3	0	0	0	0
2015	3	1	1	0	0	0	0

Namely, the most usual punishment was imprisonment of 3–6 months, twice in 2009 and 2014 and once in 2007, 2011, and 2012. Imprisonment of six months to one year was imposed twice in 2007 and once in 2012 and 2013. The most severe punishment of imprisonment of 2–3 years was imposed only once, in 2012 and 2015. It is interesting that the imprisonment of 1–2 years, as well as imprisonment of 2–3 months, were not imposed at all.

Even though imprisonment verdicts in the Criminal Code of Serbia against perpetrators of offences against human health are imposed in the criminal policy of courts (or the policy of imposing criminal sanctions), there are other kinds of criminal sanctions. Therefore, the courts punished the perpetrators of medical malpractice by fine and by probation.

It is interesting to mention the fact that in jurisdictional statistics there is no data on imposed security measures which could serve as punishment for perpetrators of this crime, such as: 1) prohibition of performing their jobs, activities, duties, and 2) deprivation of objects, nor is there any data on imposed measures of impounding the property gained by performing the crime (Table 7).

Although the punishment for medical malpractice is imprisonment, regulated in the Criminal Code in jurisdictional practice observed in the period of 2006–2015, it can be concluded that the perpetrators were punished in other ways. So, based on the analyzed data for this crime, we can conclude the following:

1) A warning issued by the court, corrective measures (which is expected since underage individuals cannot be considered to be the perpetrators a crime), an additional fine, as well as exculpation supported by law, were not imposed at all,

2) Payment of a fine as the main punishment was imposed rarely – only once in 2006 and 2015, and as many

as three times in 2012, while in the other analyzed years this punishment was not imposed on the perpetrator, and

3) Probation was the most common punishment (apart from imprisonment). It was present in every year, but imposed differently, at least once in 2006 and 2015, five times in 2007 and 2011, and as many as six times in 2013.

CONCLUSION

Among the crimes against human welfare (so-called offences against human health) there is a crime which by its significance, nature, characteristics, the perpetrator, the type, and scope of the caused consequence is singled out from Article 251 of the Criminal Code of Serbia, and it is medical malpractice. In the protection of human health, medical assistance or care for the ill has special significance and role. Individuals who are authorized to provide this very kind of assistance – medical doctors or other health workers, apart from the appropriate professional education, should act responsibly in accordance with the regulations of the medical profession, science, and skill. Hence, there is the need for stronger criminal relief for the ill. Moreover, in legal theory there are conceptions that this is the case of professional crime, or the crime of professional negligence.

The crime from Article 251 of the Criminal Code of the Republic of Serbia, under the name of medical malpractice, not only consists of violation of the rules for treating an ill individual by a doctor of medicine or dentistry, but also of illegal behavior of medical practitioners while performing any medical assistance. This crime consists of irresponsible medical assistance or care provided by a doctor or some other health practitioner which results in health deterioration of an individual.

From the conducted empirical research on the extent, the structure, dynamics, criminal prosecution policy or criminal policy for medical malpractice, which is based on statistical data in Serbia collected in the 2006–2015 period, we can conclude the following:

1) In comparison to the total number of crimes performed in Serbia, which was 108,750 in 2015, 74,279 in 2010, the highest number of crimes against health was in 2008 – 4,895, while the lowest number of these crimes was committed in 2014 – only 3,161. Health crimes are a part of crime in general, with a small share of only 5.46% in 2010 (when the maximum share was noted), or 4.81% in 2008, to the smallest share of these crimes in crime in general, in 2015, with only 3.41% and 3.43% in 2014 and 2015, respectively.

2) Medical malpractice was mostly present in 2014 and 2015, with 87 101 crimes, respectively; these were the years when the biggest share of these crimes was noted: in 2014 and 2015 the share was 2.75% and 2.71%, respectively. The crime was least present in 2011, with only 39 crimes, while the percentage was 1.02% in 2009 and 1.08% in 2008.

3) Considering the policy of criminal prosecution of this crime's perpetrator, we can say that the investigation was never interrupted, while a slightly higher number of terminations of investigation existed, supported legally. Hence, the investigation for this crime was mostly terminated in 2007, with 9.41% of the cases, and in 2006, with 8.82% of the cases (approximately every 10th report of the crime was ended by termination). However, it is obvious that there were many rejected reports. Moreover, reports for this crime were rejected mostly in 2015 – 88.12% (89 out of 101 received reports were rejected). The number of rejected reports was more than one half of the received reports, except in 2012, when "only" 34.48% of the reports (approximately one third) were rejected, or in 2006, when 45.59% of the reports were rejected.

4) Upon analyzing the ways of dealing with the reports, it can be seen that the number of investigation terminations from the legal aspects is insignificant because it varies from the minimum 0.99% in 2015 and 1.72% in 2012 to the maximum of 13.21% in 2008 and 10.59% in 2008. However, the number of individuals who were legally exculpated ranges from the minimum of 2.13% in 2010 to 19.56% in 2009, or 18.87% in 2008. It is similar with the individuals who had been reported but the report was rejected. This number goes from the minimum of 0.99% in 2015 or 2.56% in 2011 to the maximum of 13.16% in 2013.

5) While analyzing the ratio of the reported, accused, and sentenced individuals for medical malpractice, we can see that the least of them were accused in 2015 – only 14.85% (roughly every sixth reported perpetrator), or in 2006 – 19.12% out of the total percentage of the reported individuals, while the highest percentage of the sentenced individuals (56.58%) was in 2013. Regarding the number of the sentenced individuals, in comparison to the number of the reported, the highest percentage of sentenced individuals (approximately every sixth out of the reported) was 15.38% in 2011, or 15.22% in 2012 and 2007. Furthermore, a very small share of the sentenced for this crime was in 2006 and 2015, with 2.94% and 2.97%, respectively.

6) Even though this crime demanded a punishment of imprisonment, it was rarely imposed. Reportedly, in most of the cases, the punishment was imprisonment for 3–6 months, which happened twice in 2009 and 2014, and once in 2007, 2011, and 2012. This is followed by imprisonment from six months to one year, which was the sentence twice in 2007 and once in 2012 and 2013. The gravest punishment is imprisonment for 2–3 years and it was imposed only once in 2012 and 2015. Interestingly, imprisonment that ranges 1–2 years, as well as imprisonment for 2–3 months, was never imposed.

7) Other punishments were imposed as well. Warnings issued by courts, corrective measures, additional fines, and legally supported exculpation were never imposed; a fine as the main punishment was imposed rarely – only once in 2006 and 2015, and up to three times in 2012 – while the other analyzed years did not show the occurrence of this punishment. Finally, probation was the most frequent type of punishment (apart from prison); it occurred every year – at least once in 2006 and 2015, to up to five times in 2007 and 2011, and as many as six times in 2013.

Conflict of interest: None declared.

REFERENCES

1. Official Gazette of the Republic of Serbia No. 85(2005), 88 (2005), 107 (2005), 72 (2009), 111 (2009), 121 (2012), 104 (2013), 108 (2014) and 94 (2016)
2. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 490 (2009)
3. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 502 (2009)
4. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 514 (2010)
5. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 529 (2010)
6. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 546, (2011)
7. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 558, (2012)
8. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 576, (2013)
9. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 588, (2014)
10. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 603 (2015)
11. Statistical Office of the Republic of Serbia, Monthly Statistical Bulletin, Belgrade, No. 617 (2016)

Кривична одговорност за медицинску грешку у судској пракси Србије

Владимир Милетић

Први основни суд у Београду, Београд, Србија

САЖЕТАК

У области заштите и побољшања здравља људи постоји посебан значај законитог, ефикасног, редовног, професионалног и правовременог пружања медицинске заштите, обављања других здравствених услуга, или једноставно пружања медицинске помоћи или неге. На тај начин се остварује битна друштвена функција, као и заштита Уставом прокламованог права на физички и ментални интегритет људи. Међутим, погоршање здравља појединца услед медицинске грешке је могуће у процесу пружања медицинске помоћи или помоћи било које друге здравствене делатности.

Ако се ради о грубој медицинској грешци, непрописном понашању било које друге врсте или грубом кршењу правила струке, због чега постоји могућност погоршања здравља појединца или више особа, онда постоји кривично дело медицинске грешке за које закон предвиђа одговорност и кажњивост. Овај чланак говори о теоријском и практичном значају, друштвеној опасности и распрострањености овог кривичног дела и о казненој политици судова у Републици Србији, поред многих чланака у писаним и електронским медијима који изазивају велику пажњу јавности и грубе коментаре.

Кључне речи: здравље; кривично дело; одговорност; кривична санкција; политика кривичног гоњења; казнена политика судова

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине - насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5.000 речи, а за претходно и кратко саопштење, приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у СА се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и претплатници на часопис за годину у којој се рад предаје Уредништву, или измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3.000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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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.

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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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ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

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