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CONTENTS / SADRŽAJ

Pierre Fauchard (1678-1761) was a French physician who is considered the "Father of modern dentistry". He is widely known for writing the first complete scientific dental textbook entitled *Le Chirurgien dentiste*, published in 1728.

Pjer Fošar (1678-1761), francuski lekar, koji se smatra „ocem moderne stomatologije". Poznat je širom sveta kao pisac prvog kompletnog naučnog dela o stomatologiji *Le Chirurgien dentiste,* koje je objavljeno 1728. godine.

ORIGINAL ARTICLES

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Peripapillary retinal nerve fiber layer thickness in different glaucoma stages measured by optical coherence tomography

Debljina peripapilarnih retinalnih nervnih vlakana kod različitih stepena glaukoma merena optičkom koherentnom tomografijom

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Abstract

Background/Aim. One of the most reliable methods for structural measurements of glaucomatous damage is spectral domain optical coherence tomography (SD-OCT). The aim of this study was to measure peripapillary retinal nerve fiber layer (RNFL) thickness with SD-OCT in eyes with different stages of glaucoma, as well as to determine which sector in the peripapillary circle is the most vulnerable to glaucomatous damage. **Methods.** The study included 153 eyes of 93 patients with confirmed primary open angle glaucoma (POAG). All the patients underwent a complete ophthalmic examination, including visual field testing and peripapillary RNFL thickness measured by SD-OCT. They were divided into three subgroups: early, moderate and severe stage of glaucoma based on the mean deviation (MD) index of visual field. The results were presented as mean RNFL thickness: total, in the four quadrants and 12 clock-hour RNFL thickness. **Results.** The overall mean peripapillary RNFL was 74.95 ± 14.51 μm. The lower quadrant had the thickest RNFL (92.78 ± 25.84 μm), followed by upper (88.82 ± 22.04 μm), nasal (64.31 ± 11.67 μm) and temporal ones (54.02 ± 12.76 μm), showing a significant difference (χ^2 = 273.36, DF = 3, $p < 0.001$). Comparison between RNFL thickness in early glaucoma and moderate and severe stages revealed that the most sensitive sectors were inferior and superior ones, as well as sectors at 5–7 clock hour position. The greatest decrease in RNFL thickness was observed in the 9 o'clock hour sector in all three glaucoma subgroups (46.99 ± 13.28 μm), while the RNFL was the thickest in the 6 o'clock hour sector (102.63 ± 34.12 μm). **Conclusion.** Peripapillary RNFL thickness is inversely proportional to the degree of glaucomatous damage: the greater the damage, the thinner peripapillary RNFL.

Key words:
glaucoma, open-angle; disease progression; nerve fibers; optic disk; diagnosis; tomography, optical, coherence; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Jedna od najpouzdanijih metoda za merenje strukturnih promena kod glaukoma je spektralna optička koherentna tomografija – *spectral domain optical coherence tomography* (SD-OCT). Cilj rada bio je da se izmeri debljina peripapilarnih retinalnih nervnih vlakana (RNFL) uz pomoć SD-OCT, kao i da se utvrdi koji je sektor u pomenutom prostoru najosetljiviji na glaukomatozno oštećenje. **Metode.** U studiju je bilo uključeno ukupno 153 očiju 93 bolesnika sa potvrđenim primarnim glaukomom otvorenog ugla (POAG). Svim bolesnicima urađen je kompletan oftalmološki pregled, uključujući dodatno kompjuterizovano vidno polje i merenje peripapilarnih RNFL pomoću SD-OCT. Bolesnici su bili podeljeni u tri podgrupe: rani, srednji i odmakli stepen glaukoma (prema vrednostima MD na vidnom polju). **Rezultati.** Rezultati su predstavljeni kao srednje vrednosti ukupne debljine RNFL za celu grupu i podgrupe, kao i preko 4 cirkularna i 12 linearnih sektora. Srednja vrednost RNFL iznosila je 74,95 ± 14,51 μm. Najdeblji je bio donji kvadrant (92,78 ± 25,84 μm), potom gornji (88,82 ± 22,04 μm), nazalni (64,31 ± 11,67 μm) i temporalni (54,02 ± 12,76 μm) što je značajna razlika (χ^2 = 273,36, DF = 3, p < 0,001). Poređenjem debljine RNFL kod ranog glaukoma u odnosu na srednji i odmakli stadijum, najsenzitivniji su bili gornji i donji kvadrant kao i sektor od 5 do 7 sati. Najtanja je bila pozicija na 9 sati (46,99 ± 13,28 μm), a najdeblja na 6 sati (102,63 ± 34,12 μm). **Zaključak.** Debljina peripapilarnih RNFL i stepen glaukoma su obrnuto proporcionalni: veći stepen oštećenja podrazumeva tanja peripapilarna RNFL.

Ključne reči:
glaukom, otvorenog ugla; bolest, progresija; nervna vlakna; optički disk; dijagnoza; tomografija, optička, koherentna; osetljivost i specifičnost.

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Introduction

Glaucoma is a progressive multifactorial optic neuropathy characterized by structural changes of the optic nerve head (ONH) and peripapillary retinal nerve fiber layer (RNFL) damage associated with functional visual field (VF) defects. An early detection and follow up of glaucoma require functional testing using standard automated perimetry (SAP) as gold standard, particularly the 24–2 Swedish Interactive Threshold Algorithm (SITA) strategy, as well as structural testing which can be based on ophthalmic findings and followed by stereoscopic photography of ONH. But, one of the most reliable methods for objective and precise structural measurements of glaucomatous damage is the optical coherence tomography (OCT) which provides both quantitative and qualitative measurements of the RNFL thickness.

OCT in diagnostics of the ONH structural changes became a part of standard procedure for diagnosis and monitoring of patients with retinal pathology. OCT is also highly sensitive in differentiating glaucomatous from non-glaucomatous ONH changes which include (non)arteritic anterior ischemic opticopathy, intracranial tumors, optical neuritis, dominant optic atrophy, methanol poisoning, Leber's opticopathy [1–5] where OCT finding shows more diffuse decrease in peripapillary RNFL thickness in comparison to glaucoma [6]. Also, the differences between healthy eyes and eyes with glaucoma are significant [7]. Due to its advantages in performing examinations, OCT method can significantly facilitate differentiation of ONH structural damages in suspected cases.

It should be noted that numerous factors, such as age, axial length etc. can affect RNFL thickness giving false positive results shown as linear regression [8–13].

The aim of this study was to measure peripapillary RNFL thickness with spectral domain OCT (SD-OCT) in the eyes with different stages of glaucoma, as well as to determine which sector in the peripapillary circle is the most vulnerable one to glaucomatous damage.

Methods

This study included 153 eyes of 93 patients with confirmed primary open angle glaucoma (POAG). Patients with glaucoma were referred from the glaucoma department of the University Eye Clinic in Niš, Serbia. All the patients underwent complete ophthalmic examination, including best corrected visual acuity, intraocular pressure measurement by applanation tonometry, gonioscopy, visual field testing using the 24-2 SITA algorithm (Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA), slit lamp examination of anterior segment and fundus examination with a plus 90-diopter lens. Peripapillary, RNFL thickness was measured using a glaucoma analysis mode in Cirrus HD OCT device software version 6.0 (Carl Zeiss Meditec, Inc.). This study followed the tenets of the Declaration of Helsinki, the study protocol was approved by the Ethics Committee of the Medical faculty of Niš and informed consent was obtained from all the participants. They were classified into three subgroups on the basis of mean deviation (MD) index of VF: an early glaucoma (MD ≤ -6 dB), moderate glaucoma (MD between -6 dB up to -12 dB) and severe glaucoma with MD more than -12 dB. The patients with any other intraocular disease, opacification of ocular media, intraocular surgery, ocular trauma and secondary glaucoma were excluded from the study.

Inclusion criteria were: confirmed POAG (glaucomatous VF loss consistent with optic nerve damage), VF test reliability indices values, such as false positive, false negatives and fixation loss less than 20%; no ocular opacities or other ocular pathology, no other structural optic nerve abnormalities or secondary glaucoma; negative history of previous eye disease, trauma and/or eye surgery and no neurologic disease; axial length in referral values.

ONH imaging was automatically made over an area of 6×6 mm by a 200×200-pixel resolution axial scan. Each eye was dilated with tropicamide 1% drops before recording. Images with a signal power more than seven were used for analysis. In all subgroups overall mean of the whole circle circumference, linear maps at 12 o'clock hour positions and circular maps within 4 quadrants and mean peripapillary RNFL thickness were recorded for each patient.

For the statistical analysis, mean peripapillary RNFL and its segment in four quadrants, as well as at 12 o'clock hour positions were calculated. Comparison between the means of the groups for paired variables was evaluated by one-way ANOVA. Kolmogorov-Smirnov test was used to determine whole circle circumferences, 4 quadrants and 12 clock-hour sectors RNFL thickness distribution in the observed group and subgroups. Data were analyzed using SPSS v. 20.0 for Windows (SPSS, Inc., Chicago, IL). The value of $p < 0.05$ was considered statistically significant.

Results

The study included 153 randomly selected eyes in 93 POAG patients (52 female and 41 male), mean age of 65.09 ± 10.12 years (range 20–59 years). The subgroups were age and sex matched. The overall mean peripapillary RNFL was 74.95 ± 14.51 μm. The RNFL was the thickest in the lower quadrant (92.78 μm), followed by upper (88.82 μm), nasal (64.31 μm), and temporal (54.02 μm) quadrant. The overall mean, 4 quadrants mean, and 12 clock-hour sectors mean RNFL thiakness values are shown in Table 1.

There was a highly significant difference between the observed quadrants in different glaucoma stages (χ^2 = 273.36, DF = 3, $p < 0.001$). Temporal quadrant was highly significantly thinner than all the other quadrants, while the nasal one was significantly thinner than upper and lower ones ($p < 0.001$). A detailed comparison of the peripapillary RNFL thickness in all four sectors and among different stages of glaucoma showed the following results: in temporal and nasal sectors there was no difference in RNFL thickness regression between moderate and severe glaucoma (the loss of RNFL thickness was 17% *vs* 8%, respectively). But, when we calculated the RNFL thickness in moderate and severe glaucoma in comparison to early glaucoma measurements, we found the RNFL thickness

Table 1

Peripapillary retinal nerve fiber layer (RNFL) thickness measured by spectral domain optical coherence tomography (OCT) – SD OCT (overall, four quadrants and 12 o'clock sectors)

Parameter	RNEL thickness (μm) [x̄ ± SD; med (range)]							
	Total (n = 153 eyes)		Glaucoma mild (n = 74 eyes)		Glaucoma moderate (n = 36 eyes)		Glaucoma severe (n = 43 eyes)	
Overall mean	74.95 ± 14.51	77.00 (45–105)	83.60 ± 9.45	84.00 (60–105)	70.97 ± 12.88	70.00 (53–101)	63.40 ± 13.65	62.00 (45–105)
Mean by quadrant								
superior	88.82 ± 22.04	90.00 (45–144)	101.03 ± 16.14	98.00 (66–144)	83.42 ± 18.12	83.00 (54–127)	72.35 ± 21.58	68.00 (45–131)
nasal	64.31 ± 11.67	63.00 (41–109)	66.92 ± 11.98	65.50 (46–109)	62.06 ± 11.08	60.00 (45–101)	61.70 ± 10.85	60.00 (41–85)
inferior	92.78 ± 25.84	96.00 (44–144)	107.51 ± 17.54	110.00 (68–144)	89.97 ± 24.99	87.00 (56–135)	69.79 ± 20.74	63.00 (44–143)
temporal	54.02 ± 12.76	52.00 (32–100)	59.01 ± 12.27	54.50 (34–100)	48.78 ± 10.41	47.00 (32–71)	49.81 ± 12.34	46.00 (32–82)
Mean by 12 o'clock sectors								
1	86.61 ± 27.12	80.00 (40–155)	98.01 ± 25.77	98.00 (44–155)	79.94 ± 22.46	76.00 (42–128)	72.56 ± 24.85	67.00 (40–149)
2	71.94 ± 16.39	68.50 (46–140)	79.58 ± 17.69	75.00 (53–140)	65.30 ± 9.27	66.00 (49–83)	67.06 ± 14.86	61.50 (46–112)
3	57.54 ± 10.86	57.00 (33–88)	57.77 ± 12.08	58.00 (33–88)	55.04 ± 9.02	55.00 (41–70)	58.86 ± 10.41	58.50 (36–81)
4	60.62 ± 11.17	59.50 (38–98)	61.95 ± 12.07	60.00 (38–98)	58.00 ± 11.02	57.00 (42–88)	60.69 ± 10.11	61.00 (39–82)
5	88.50 ± 30.32	82.00 (38–200)	102.03 ± 27.77	102.50 (47–176)	83.72 ± 29.45	76.00 (38–200)	69.21 ± 23.24	62.00 (43–146)
6	102.63 ± 34.12	102.00 (44–181)	119.19 ± 25.79	118.00 (60–181)	102.00 ± 36.28	97.00 (55–169)	74.65 ± 25.98	67.00 (44–167)
7	87.01 ± 30.00	79.00 (35–171)	101.15 ± 27.44	99.00 (53–171)	84.57 ± 29.59	76.00 (48–157)	64.98 ± 19.13	59.00 (35–136)
8	55.63 ± 15.90	52.50 (27–111)	63.02 ± 16.70	62.00 (33–111)	47.09 ± 12.96	45.00 (28–75)	52.25 ± 12.75	52.00 (27–76)
9	46.99 ± 13.28	44.00 (28–100)	49.65 ± 13.07	47.00 (29–100)	40.70 ± 11.14	39.00 (29–81)	47.83 ± 13.80	47.50 (28–83)
10	60.53 ± 18.44	57.50 (32–130)	71.91 ± 18.32	68.00 (40–130)	53.83 ± 14.03	51.00 (34–81)	51.22 ± 13.30	48.50 (32–83)
11	88.00 ± 26.00	86.00 (41–163)	100.20 ± 22.21	97.50 (42–163)	84.11 ± 25.80	78.50 (49–150)	70.12 ± 20.80	70.00 (41–141)
12	91.92 ± 28.38	87.00 (43–166)	105.08 ± 25.06	107.00 (55–166)	86.11 ± 22.68	82.50 (60–149)	74.14 ± 27.15	63.00 (43–156)

x̄ – mean value; SD – standard deviation; med – median

loss of by 18% in the superior sector compared to thickness in early glaucoma, as well as more thinning in severe glaucoma, up to 29%, compared to early glaucoma thickness. In inferior sector, results of peripapillary RNFL show thinning of 16% in moderate, up to 35% in severe glaucoma.

The curves of RNFL thickness in three glaucomatous subgroups are presented in Figure 1. As it can be seen, the thinnest sector in all the three glaucoma subgroups (mean thickness was 46.99 ± 13.28 μm) is at 9 o'clock hour position. The RNFL thicknesses among the different glaucoma subgroups was significantly different, but an overlapping was observed. At positions 3, 4, 8 and 9 o'clock, the thinnest peripapillary RNFL was in moderate glaucoma instead of severe glaucoma, as expected. It is interesting that at positions from 3 up to 5 o'clock, there was no difference in RNFL thickness with repeated overlapping in moderate glaucoma subgroup vs. severe glaucoma subgroup.

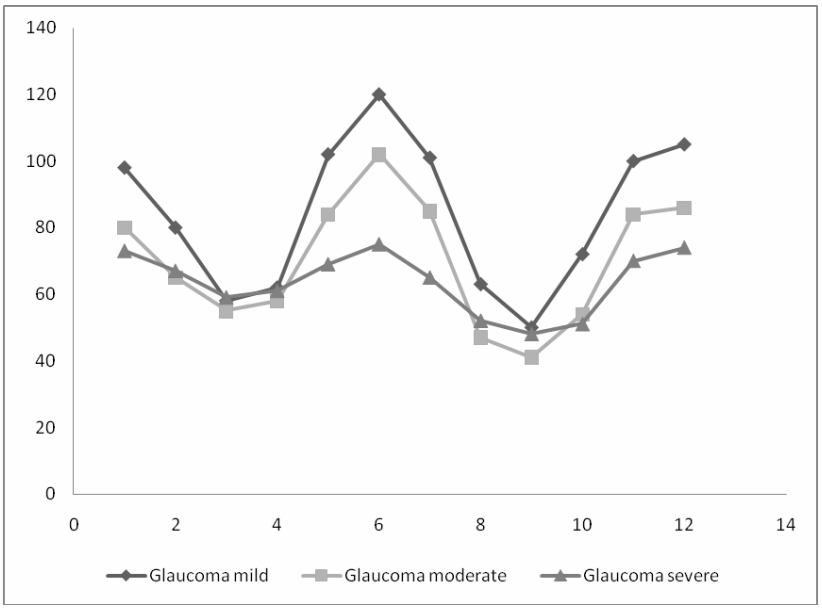

Fig. 1 – Thickness (μm) of peripapillary retinal nerve fiber layer (RNFL) in early (mild), moderate and severe glaucoma in 12 o'clock sectors.

The results of this study show that the greatest thinning and linear regression of peripapillary RNFL from early to moderate and, finally, severe glaucoma, were in the following positions: 6 o'clock position – in moderate glaucoma 15% up to 38%, at 7 o'clock position - 16% up to 37%, at 5 o'clock hour – 18% up to 33%.

Discussion

OCT offers a possibility of quantitative and morphologic RNFL analysis. For the purposes of measuring peripapillary RNFL thickness in this study we used Cirrus HD-OCT, which was found to be a reliable diagnostic tool with excellent reproducibility regarding the monitoring of glaucoma progression, as demonstrated by many studies [14, 15]. Also, SD-OCT demonstrates advantage over time domain OCT for two reasons. First, it shows an enlarged circle around the optic nerve head with sufficient detail to identify possible errors caused by influence of potential epiretinal membrane, vitreous detachments, etc. Second, high-quality scans allow detailed visualization [16].

Basic facts about optic nerve fiber layer thickness are the following: average RNFL thickness represents the mean thickness of the entire nerve fiber that reaches the optic disc. Also, the majority of the nerve fibers converge on the optic disc either superiorly or inferiorly. In the present study, the peripapillary RNFL was thicker superiorly and inferiorly, which can be explained by normal peripapillary RNFL distribution. In early glaucoma, as reported in some histological studies, there is 25–35% loss of retinal ganglion cells before VF damage is confirmed, while many of these nerve fibers are still undamaged [17]. Taliantzis et al. [18] reported that deep structural alterations detected by OCT are an important indicator of early glaucomatous changes, even if they are not detected with SAP. Once the VF loss is established, smaller amounts of RNFL thickness are necessary for the reduction of mean deviation value [19].

In our study, the inferior RNFL thickness shows the highest thinning in advanced glaucoma eyes, what is in correlation with the results of some previous studies [20]. Peripapillary, RNFL demonstrated higher sensitivity and specificity than macular RNFL for glaucomatous and suspect groups and even then, the inferior quadrant is the most sensitive parameter [21]. Mean RNFL thickness in inferior and superior quadrants, as well as 6, 7, 11 and 12 clock hour segment thickness have the highest sensitivity and specificity in distinguishing eyes with glaucomatous VF defects from normal eyes [22]. Hwang and Kim [23] reported that the eyes with more advanced glaucoma had thinner RNFL in global area, superior, inferior and temporal quadrants. All the mentioned studies report similar results, as we mentioned in this study. A significant difference in superior and inferior peripapillary quadrants between glaucomatous and normal eyes was detected by scanning laser polarimetry, as Galväo Filho et al. [19] reported as well. The authors found out a significant difference in RNFL thickness between healthy eyes and eyes of

those with early glaucoma, but not between early and moderate glaucomatous eyes. Our study shows that the linear reduction of RNFL thickness as glaucomatous damage is more progressive. It should be mentioned that more glaucomatous eyes were included in the study (153 *vs* 68) and we used SD OCT, not scanning laser polarimetry for RNFL thickness measurement. However, even a complete loss of ganglion cells, as it is demonstrated in severe glaucoma, leaves some residual thickness which consists of glial cells and blood vessels [24]. In the end, even residual retinal thickness in the eyes with severe glaucoma is different in different peripapillary sectors, corresponding with papillomacular bundle. It supports the result of this study that in nasal and temporal sectors there is no difference between different glaucoma stages, while in superior and inferior quadrants the thinning of peripapillary RNFL is significant.

As previously mentioned, SD-OCT is useful for determining structural changes in peripapillary RNFL thickness, and also for evaluating the correlation between perimetric defects and corresponding nerve fiber loss [25]. New summary statistics combining VF and OCT results are being developed, as it has been reported in a study of Racette et al. [26] who integrated functional and structural measurements using Artificial Neural Networks. Very similar to these results, Bizios et al. [27] demonstrate relevance vector machine on a combined optimized confocal scanning laser ophthalmology (CSLO) device using the Heidelberg retina tomograph (HRT) and short-wavelength automated perimetry data for the purpose of improving glaucoma diagnostic accuracy. The multivariate and Moorfields algorithm of the HRT provide good ability to distinguish glaucoma tous from normal eyes [22].

Most studies included patients with VF loss, as we did. But, when glaucoma suspects with normal VF were included in the study, the sensitivity and specificity of diagnostic tests were significantly lower when compared to cases with glaucomatous VF abnormalities [22]. In presented study we were focused on a SD OCT potential and power to detect the difference between RNFL thickness in different glaucoma stages without comparing with VF defects. Since the before mentioned approach could have limited utility, we considered that it is very important to define the peripapillary RNFL sectors that are most vulnerable to glaucomatous damage.

As it was reported, a simple OCT imaging report is sensitive to providing quantitative information for most cases, but for difficult ones interpreting physicians should have considerable experience in analyzing OCT scans [16] and they should always have on mind that OCT is only a part of the glaucoma diagnostics and the following up process which is of great importance when combined with SAP or CSLO results.

Conclusion

The sector of the thickest peripapillary RNFL is the temporal one, followed by nasal, superior and inferior sectors. The thinning of the RNFL at advanced stages of glaucoma is greater in the inferior and superior sectors. There is no decrease of peripapillary RNFL thickness in the nasal and temporal quadrants in comparison to early glaucoma RNFL thickness. The most valuable and sensitive parameters in assessing the degree of peripapillary RNFL glaucomatous damage are in the 5, 6 and 7 clock hour sectors.

REFERENCES

1. *Deleon-Ortega JE, Arthur SN, McGwin G Jr, Xie A, Monheit BE, Girkin CA.* Discrimination between glaucomatous and nonglaucomatous eyes using quantitative imaging devices and subjective optic nerve head assessment. Invest Ophthalmol Vis Sci 2006; 47(8): 3374−80.
2. *Medeiros FA, Zangwill LM, Bowd C, Vessani RM, Susanna R, Weinreb RN.* Evaluation of retinal nerve fiber layer, optic nerve head, and macular thickness measurements for glaucoma detection using optical coherence tomography. Am J Ophthalmol 2005; 139(1): 44−55.
3. *Jaffe GJ, Caprioli J.* Optical coherence tomography to detect and manage retinal disease and glaucoma. Am J Ophthalmol 2004; 137(1): 156−69.
4. *Greaney MJ, Hoffman DC, Garway-Heath DF, Nakla M, Coleman AL, Caprioli J.* Comparison of optic nerve imaging methods to distinguish normal eyes from those with glaucoma. Invest Ophthalmol Vis Sci 2002; 43(1): 140−5.
5. *Zangwill LM, Bowd C, Berry CC, Williams J, Blumenthal EZ, Sánchez-Galeana CA,* et al. Discriminating between normal and glaucomatous eyes using the Heidelberg Retina Tomograph, GDx Nerve Fiber Analyzer, and Optical Coherence Tomograph. Arch Ophthalmol 2001; 119(7): 985−93.
6. *Gupta PK, Asrani S, Freedman SF, El-Dairi M, Bhatti TM.* Differentiating glaucomatous from non-glaucomatous optic nerve cupping by optical coherence tomography. Open Neurol J 2011; 5: 1−7.
7. *Cvenkel B, Kontestabile AS.* Correlation between nerve fibre layer thickness measured with spectral domain OCT and visual field in patients with different stages of glaucoma. Graefes Arch Clin Exp Ophthalmol 2011; 249(4): 575−84.
8. *Sakata LM, Deleon-Ortega J, Sakata V, Girkin CA.* Optical coherence tomography of the retina and optic nerve: A review. Clin Experiment Ophthalmol 2009; 37(1): 90−9.
9. *Savini G, Barboni P, Carbonelli M, Zanini M.* The effect of scan diameter on retinal nerve fiber layer thickness measurement using stratus optic coherence tomography. Arch Ophthalmol 2007; 125(7): 901−5.
10. *Parikh RS, Parikh SR, Sekhar GC, Prabakaran S, Babu JG, Thomas R.* Normal age-related decay of retinal nerve fiber layer thickness. Ophthalmology 2007; 114(5): 921−6.
11. *Wu Z, Vazeen M, Varma R, Chopra V, Walsh AC, LaBree LD,* et al. Factors associated with variability in retinal nerve fiber layer thickness measurements obtained by optical coherence tomography. Ophthalmology 2007; 114(8): 1505−12.
12. *Harwerth RS, Wheat JL, Rangaswamy NV.* Age-related losses of retinal ganglion cells and axons. Invest Ophthalmol Vis Sci 2008; 49(10): 4437−43.
13. *Shoji T, Sato H, Ishida M, Takeuchi M, Chihara E.* Assessment of glaucomatous changes in subjects with high myopia using spectral domain optical coherence tomography. Invest Ophthalmol Vis Sci 2011; 52(2): 1098−102.

14. *Soltani-Moghadam R, Alizadeh Y, Kazemnezhad Leili E, Ahsari Haghighi M*. Reproducibility of peripapillary retinal nerve fiber layer thickness measurements with cirrus HD-OCT in glaucomatous eyes. Int J Ophthalmol 2015; 8(1): 113−7.
15. *Mwanza J, Chang RT, Budenz DL, Durbin MK, Gendy MG, Shi W,* et al. Reproducibility of peripapillary retinal nerve fiber layer thickness and optic nerve head parameters measured with cirrus HD-OCT in glaucomatous eyes. Invest Ophthalmol Vis Sci 2010; 51(11): 5724−30.
16. *Hood DC, Raza AS*. On improving the use of OCT imaging for detecting glaucomatous damage. Br J Ophthalmol 2014; 98(Suppl 2): ii1−ii9.
17. *Kerrigan-Baumrind LA, Quigley HA, Pease ME, Kerrigan DF, Mitchell RS*. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. Invest Ophthalmol Vis Sci 2000; 41(3): 741−8.
18. *Taliantzis S, Papaconstantinou D, Koutsandrea C, Moschos M, Apostolopoulos M, Georgopoulos G*. Comparative studies of RNFL thickness measured by OCT with global index of visual fields in patients with ocular hypertension and early open angle glaucoma. Clin Ophthalmol 2009; 3: 373−9.
19. *Galvão Filho RP, Vessani RM, Susanna R Jr*. Comparison of retinal nerve fibre layer thickness and visual field loss between different glaucoma groups. Br J Ophthalmol 2005; 89(8): 1004−7.
20. *Sihota R, Sony P, Gupta V, Dada T, Singh R*. Diagnostic capability of optical coherence tomography in evaluating the degree of glaucomatous retinal nerve fiber damage. Invest Ophthalmol Vis Sci 2006; 47(5): 2006−10.
21. Leung CK, Chan WM, Yung WH, *Ng AC*, *Woo J*, *Tsang MK*, et al. Comparison of macular and peripapillary measurements for the detection of glaucoma: An optical coherence tomography study. Ophthalmology 2005; 112(3): 391−400.
22. *Lin SC, Singh K, Jampel HD, Hodapp EA, Smith SD, Francis BA*, et al. Optic nerve head and retinal nerve fiber layer analysis: a report by the American Academy of Ophthalmology. Ophthalmology 2007; 114(10): 1937−49.
23. *Hwang YH, Kim YY*. Glaucoma diagnostic ability of quadrant and clock-hour neuroretinal rim assessment using cirrus HD optical coherence tomography. Invest Ophthalmol Vis Sci 2012; 53(4): 2226−34.
24. *Hood DC, Anderson SC, Wall M, Kardon RH*. Structure versus function in glaucoma: an application of a linear model. Invest Ophthalmol Vis Sci 2007; 48(8): 3662−8.
25. *Horn FK, Mardin CY, Laemmer R, Baleanu D, Juenemann AM, Kruse FE,* et al. Correlation between local glaucomatous visual field defects and loss of nerve fiber layer thickness measured with polarimetry and spectral domain OCT. Invest Ophthalmol Vis Sci 2009; 50(5): 1971−7.
26. *Racette L, Chiou CY, Hao J, Bowd C, Goldbaum MH, Zangwill LM,* et al. Combining functional and structural tests improves the diagnostic accuracy of relevance vector machine classifiers. J. Glaucoma 2010; 19(3): 167−75.
27. *Bizios D, Heijl A, Bengtsson B*. Integration and fusion of standard automated perimetry and optical coherence tomography data for improved automated glaucoma diagnostics. BMC Ophthalmol 2011;11: 20.

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The importance of impulse oscillometry in bronchial provocation testing in confirming the diagnosis of asthma in male army recruits

Značaj impulsne oscilometrije kod bronhoprovokativnog testiranja za potvrdu dijagnoze astme kod muških vojnih regruta

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Abstract

Background/Aim. Impulse oscillometry (IOS) is a technique valid for measuring the lung function in obstructive lung diseases and bronchial provocation tests. However, no consensus exists for its use. The aim of the study was to assess impulse oscillometry sensitivity for detection of early airways changes during bronchial provocation testing and to compare with changes obtained with spirometry and bodyplethysmography in male army recruits. **Methods.** Male military recruits were submitted to bronchial provocation test with histamine by the aerosol provocation system. Out of 52 male military recruits subjected to attempts to make the diagnosis of asthma the study included 31 subjects with fall of forced expiratory volume in one second (FEV1) above 20%. The changes of impulse oscillometry were measured one step before and after provocation dose (PD) of histamine and compared with the changes of bodyplethysmography and spirometry. **Results.** The average age of male army recruits was 23.3 year. After bronchoprovocation there was an average increase of the total resistance at 5 Hz (R5) by 66.6%, resonant frequency (Fres) by 102.2%, Goldman index (AX) by 912.1%, the arway resistance (Raw) by 121.5%, and a decrease in reactance at 5 Hz (X5) by 132.1% and FEV1 by 25.6%. One step before the last inhaled of PD20 there was an average increase of 26.7% in R5, 24.1% in Fres, 85.3% in AX, 11.9% in Raw and a decrease in X5 by 26.9% and FEV1 by 4.3%. A correlation between impulse oscillometry and bodyplethysmography parameters was obtained. **Conclusion.** This paper demonstrates a sufficient sensitivity of impulse oscillometry to detect changes in airways, so it may play a complementary role in the diagnosis of asthma in male military recruits.

Key words:
asthma; diagnosis; bronchial provocation tests; histamine; respiratory function tests; sensitivity and specificity; military personnel; men; personnel selection.

Apstrakt

Uvod/Cilj. Impulsna oscilometrija (IOS) je važeća tehnika za merenje respiratorne impedancije kod opstruktivnih bolesti i bronhoprovokativnih testova, ali ne postoji konsenzus za njeno korišćenje. Cilj studije bio je da se proceni osetljivost impulsne oscilometrije u detekciji ranih promena u disajnim putevima za vreme bronhoprovokativnog testiranja i da se uporedi sa promenama spirometrije i pletizmografije kod muških vojnih regurta. **Metode.** Muškim vojnim regurtima je urađen bronhoprovokativni test sa histmainom preko aerosolnog provokacionog sistema. Od 52 muška vojna regruta kod kojih je pokušano potvrđivanje dijagnoze astme u studiju je bio uključen 31 ispitanik sa padom *forced expiratory volume in one second* (FEV1) iznad 20%. Merene su promene impulsne oscilometrije pre i posle pada FEV1 za 20% nakon provokacione doze (PD) histamina i upoređivane sa promenama spirometrije i telesne pletizmografije. **Rezultati.** Prosečna starost muških vojnih regruta bila je 23,3 godine. Posle bronhoprovokacije prosečno je povećan realni otpor (resistanca) na 5 herca (R5) za 66,6%, rezonantna frekvenca (Fres) za 102,2%, Goldman-ov indeks (AX) za 912,1%, endobronhijalna rezistanca (Raw) za 121,5% i smanjena reaktansa na 5 Hz (X5) za 132,1% i FEV1 za 25,6%. Korak pre inhalirane PD20 doveo je prosečno do povećanja R5 za 26,7%, Fres za 24,1%, AX za 85,3%, Raw za 11,9% i X5 za 26,9% i FEV1 od 4,3. Visok stepen korelacije dobijen je između telesne pletizmografije i IOS. **Zaključak.** U radu je dokazana dovoljna osetljivost impulsne osecilometrije za detekciju ranih promena u disajnim putevima, te ona može igrati komplementarnu ulogu u dijagnozi astme kod muških vojnih regruta.

Ključne reči:
astma; dijagnoza; inhalacioni testovi; histamin; respiratorna funkcija, testovi; senzitivnost i specifičnost; kadar, vojni; muškarci; kadar, selekcija.

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Introduction

Asthma is a chronic inflammation, associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, shortness of breath, chest tightness and coughing particularly at night or early in the morning [1]. It is characterized by reversible airflow obstruction, inflammation and hyperactivity of the airways [2].

Bronchial hyperresponsiveness (BHR) or increased sensitivity of the airways can be defined as a greater tendency to narrowing of the airways in response to inhalation of chemicals (metilcholin, histamine, carbachol) and physical agents (cold air, hyper- and hypo-osmolarity solutions), allergens, or exertional [3]. It is caused by genetic or environmental factors. Bronchial hyperresponsiveness presents the physiological trademark of asthma, and it does not involve setting diagnosis of asthma. The presence and degree of BHR is measured by standardized bronchial provocation tests (BPT). They are performed by inhalation of substances that cause narrowing of the airways and increased work of breathing. Asthma patients respond to nonspecific agents quickly and more strongly, up to 100 times higher than healthy ones. Bronchial provocation testing is usually performed with histamine as provocative substances [4, 5].

The diagnosis of asthma is a set of characteristic symptoms, skin allergy tests to inhalation allergens, total and specific immunoglobulin E (IgE), sputum eosinophilia, and demonstrate BHR with spirometry, bodyplethysmography and impulse oscillometry as a new method of measuring pulmonary function [6].

The impulse oscillometry system (IOS) is used for determining the mechanical properties of the lungs and respiratory system through the relationship of pressure (P) and flow (V) measurement of respiratory impedance (Z) over the input pulses. Z representing the interaction between the impulse of pressure, resistance and the reactivity of the respiratory system, which includes the resistance (R) and reactance (X). Resistance is the result of a mechanical breathing and airway resistance. Reactance represents a reactive resistance which is contained in that part of the lung where it is not possible to measure the real resistance, and it is on the periphery. Reactance contains two components: capacitance (C) and inertance (I). Respiratory impedance is measured by impulse oscillometry [7]. Impulse oscillometry testing does not depend on cooperation of patients, because it is perfect in pulmonology [8], pediatrics [9], geriatrics [10], occupational medicine [11], anesthesiology [12], otorinolaryngolgy [13], sports medicine [14], and experimental medicine [15]. Impulse oscillometry has demonstrated high sensitivity in the assessment of BHR at BPT in the early diagnosis of asthma [16–19].

The aim of the research was to determine the sensitivity of impulse oscillometry in the early detection of bronchial hyperreactivity during BPT and compare the parameters of IOS with the results of spirometry and bodyplethysmography in male army recruits.

Methods

Out of 52 male military recruits, subjected to establishing the diagnosis of asthma, the study included 31 subjects, aged 23.3 years in average.

Bronchial provocation test with histamine was analyzed by measuring impulse oscillometry, spirometry, and bodyplethysmography. The changes of impulse oscillometry before and after the fall in forced expiratory volume in one second (FEV1) by 20% after provocation dose (PD20), and comparisons were performed with changes in spirometry (FEV1) and bodyplethysmography endobronchial resistance (Raw), specific resistance (Sraw), and specific conductance (SGaw) [20].

Criteria for inclusion of subjects in the study were: male military recruits aged from 17 to 27 years; asthma according to medical records; indications on guidelines for BPT. Criteria for exclusion from the study were the absolute and relative contraindications to the guidelines [21].

Medical history, physical examination and measurement of lung function were performed in all the subjects. Bronchial provocation test was evaluated with impulse oscillometry, spirometry and bodyplethysmography. The basic measurements with three tests were performed before the start of BPT. Bronchial provocation testing started so that the subjects inhaled l mL of physiological saline (0.9% NaCl), and after 2 min continued with measurements of lung function then the results were compared with the results of the basic measurement, and then the test using the same model of histamine inhalation in the dose of 0.03; 0,06; 0.12; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0, and 16 mg/mL) [21, 22]. Histamine was inhaled in the form of solution of 32 mg/mL and 4 mg/mL (the Institute of Virology and Immunology, Torlak, Beograd) *via* the aerosol provocation system (APS) [23], which automatically designates the given dose.

Survey instruments for measuring lung function were pulsed oscillometer, Series Master Screen IOS (Care Fusion, Jaeger, Würtzburg, Germany) for the measurement of respiratory impedance, spirometer for measuring static and dynamic airway volume, and bodyplethysmography to determine the airway resistances and intrathoracic gas volumes were examined with Master Screen Body (Care Fusion, Jaeger, Würtzburg, Germany). Measurements of impulse oscillometry were performed as recommended by the constructor (Smith HJ), spirometry and bodyplethysmography according to the standards of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) were performed [24–26].

Impulse oscillometry was accompanied by the following parameters: total resistance at 5 Hz (R5) (kP/L/s), resistance at 20 Hz (R20) (kP/ L/s), reactance at 5 Hz (X5) (kP/l/s), resonant frequency (Fres) (L/s); Goldman index (AX); spirometry: forced vital capacity (FVC) (L), forced expiratory volume in first second (FEV1) (L), the ratio of FEV1 / FVC (%), forced expiratory flow 50% (-FEF 50) (L); body plethysmography: total endobronchial resistance-Raw (kPa.Ls-1) specific resistance-(Sraw) (kPa.s-1) and specific conductance (Sgaw) (1 / kPa.s-1). The absolute values and

their changes (%) of spirometry (FVC, FEV1, FEF50) and bodyplethysmography (Raw, SRaw, SGaw) and impulse oscillometry (R5, X5, Fers) are shown.

To calculate relative values of the parameters of impulse oscillometry (R5, X5), reference values of constructor appliances (Vogel H and Smith HJ) were used, while for spirometry (FVC, FEV 1, FEF 50) and body plethysmography (Raw, SRaw, SGaw), predicted value of the European Respiratory Society was used. Parameters Raw, SRaw and Fres are presented in absolute values. Reactance at 5 Hz was calculated as the difference between the active and planned values, the original formula produced in the software, in the literature referred to as delta X5 [27].

After collecting and controlling data, the standard statistical methods were used: absolute number, percentage, arithmetic mean, standard deviation. The frequency changes of R5, Fres and AX are shown. The degree of correlation was determined by the coefficient of linear correlation. Statistical analysis was performed on a personal computer by means of statistical software package "Statistica".

Results

Data analysis was performed in 31 male military recruits, the average age of 23.2 ± 5.3. A total of 56% were smokers and 44% non-smokers.

Spirometry was performed in all the patients. The mean basic values of FVC were 5.6 L, for FEV1, and 4.56 L, for FEF50 4.73 L. The average values of changes of FEV1 for 4.3% and FEF50 for 6.8% was decreased before PD20. The mean values of changes of FEV1 to 25.6% and 46.7% for FEF50 were obtained after PD20 (Table 1).

Body plethysmography was also done in all the subjects. The average values of Raw before BPT were 0.25 kPa-L. The mean values of changes of Raw for 11.4% and 14.9% for SRaw were increased and SGaw for 6.0% was decreased before PD20. In the control group the mean values of changes of Raw for 104.2%, SRaw for 121.5% was increased and SGaw for 47.1% was decreased.

Impulse oscillometry basic values of R5 0.34 kPa/L/s, X5 -0.09 kP/L/s, Fres 10.2 L /s and AX 0.23 were shown. The average values of changes of R5 for 26.7%, X5 for 26.9%, Fres for 24.1% and AX for 85.3% were increased before PD20. The average values of changes of R5 for 66.4%, X5 for 132.1%, Fres for 102.2% and AX for 912.1% were increased after PD20.

The values of the main parameters of impulse oscillometry R5 and Fres were followed by frequency changes before and after PD20 over four class intervals (less than 20%, 21–30%, 31–40% and over 40%), and also AX parameter (below 100 %, of 101–150%, 151–200%, more than 200%) (Table 2).

In the study group the frequency of changes of R5 under 20% of the values were 60% of the cases, in 20% from

Table 1

Descriptive statistics before and after histamine bronchoprovocation dose 20 (PD20)

Variable	Basic values	Before PD20	Δ Before PD20	After PD20	Δ After PD20
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Spirometry					
FVC	5.60 ± 0.81	5.00 ± 1.38	-1.39 ± 6.37	4.77 ± 0.88	-12.70 ± 10.08
FEV1	4.56 ± 0.64	4.47 ± 0.64	-4.30 ± 6.99	3.37 ± 0.60	-25.66 ± 11.48
FEF50	4.73 ± 1.05	4.40 ± 0.97	-6.86 ± 14.64	2.60 ± 0.86	-46.74 ± 10.10
Body plethysmography					
Raw	0.25 ± 0.07	0.27 ± 0.06	11.98 ± 32.53	0.48 ± 0.18	104.29 ± 97.32
SRaw	1.05 ± 0.34	2.11 ± 1.05	14.93 ± 31.62	1.23 ± 0.34	121.53 ± 107.32
SGaw	1.03 ± 0.32	0.86 ± 0.22	-6.02 ± 28.55	0.51 ± 0.19	-47.10 ± 20.37
Impulse oscillometry					
R5	0.34 ± 0.09	0.41 ± 0.15	26.74 ± 19.04	0.54 ± 0.17	66.64 ± 62.91
X5	-0.09 ± 0.05	-0.11 ± 0.05	26.90 ± 36.31	-0.17 ± 0.15	132.18 ± 148.13
Fres	10.27 ± 2.60	13.49 ± 4.01	24.13 ± 19.31	19.66 ± 6.96	102.22 ± 95.22
AX	0.23 ± 0.16	0.38 ± 0.29	85.30 ± 73.09	1.41 ± 1.42	912.12 ± 1422.73

FVC – forced vital capacity; FEV1 – forced expiratory volume in one second; FEF50 – forced expiratory flow 50%; Raw – endobronchial resistance; SRaw – specific resistance; SGaw – specific conductance; R5 – total resistance at 5 Hz; X5 – reactance at 5 Hz; Fres – resonant frequency; AX – Goldman index.

Table 2

Frequency of changes of impulse oscillometry (IOS) parameters before and after histamine bronchoprovocation dose 20 (PD20) for resistance at 5 Hz (R5), resonant frequency (Fres) and Goldman index (AX)

Intervals (%)	ΔR5 before PD20 (%)	ΔR5 after PD20 (%)	ΔFres before PD20 (%)	ΔFres after PD20 (%)	Intervals (%)	ΔAX before PD20 (%)	ΔAX after PD20 (%)
< 20	60.0	16.0	64.0	8.0	< 100	72.0	4.0
21–30	20.0	24.0	16.0	4.0	101–150	20.0	16.0
31–40	12.0	16.0	16.0	12.0	151–200	4.0	20.0
> 40	8.0	44.0	4.0	76.0	> 200	4.0	60.0

ΔR5 – interval of total resistance; ΔFres – interval of resonant frequency; ΔAX – interval of Goldman index.

21–30%, in 12% from 31–40% and 8% of the cases over 40% of the values. In the control group the frequency of changes under 20% ocurred in 16% of the study subjects, in 24% from 21–30%, in 16% from 31–40% and more than 40% of the values in 44% of the cases. In the group before PD20 the changes of Fres were in 64% of the subject below 20% of values, in 16% from 21–30%, in 16% from 31–40% and more than 40% of values in 4% of the cases. In the control group changes below 20% occurred in 8%, from 21–30% in 4%, from 31–40% in 12%, and more than 40% of values in 76% of the cases. In the group before PD20 change AX in 72% of the patients were below 100% of values, in 20% from 101–150%, in 4% from 151–200% and in 4% of the cases over 200% of values, while in the control group changes below 100% of values were in 4% of the subjects, from 101–150% in 16%, from 151–200% and over 200% in 16% of values in 60% of the cases (Table 2).

A correlation before PD20 between IOS and body plethysmography was weak according to spirometry (Table 3). A high degree of significant correlation after PD20 was observed between the values of IOS (Table 4) and body plethysmography, and the most pronounced between Raw and R5 (0.74), Fres (0.82) and AX (0.88).

Disscusion

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction, inflammation and hypereactivity of the airways. BHR is proving bronchodilation and bronchial provocation tests [2]. Bronchial provocation testing is performed by inhalation of substances that cause narrowing of the airways and increased work of breathing. Bronchial hyperresponsiveness does not involve setting diagnosis but it certainly is an indicator of the existence of asthma. Bronchial hyperresponsiveness is proved by default, just call spirometry and impulse oscillometry can be used as an additional method that has proven to be sensitive. Mean values of changes in the parameters of IOS before PD20 (R5 26.7%, Fres 24.1%) were 6 times greater than changes in spirometry (FEV1 4.3%) and bodyplethysmography (Raw 11.9%, SRaw 14.9%), and also after PD20 average changes of IOS (R5 66.4%, and 102.2% Fres) were higher from 2.5 to 4 times than changes of spirometry (FEV1 25.66%), which makes this method sensitive. The high variability of these parameters of IOS can create confusion over the interpretation of the findings, especially the Goldman index (AX) cannot be used for BPT

Table 3

Pearson's correlation coefficient between changes before histamine bronchoprovocation dose 20 (PD20) [impulse oscillometry (PD20-IOS) vs. spirometry, plethysmography]

Variable	ΔR5 (%) PD20	ΔX5 (%) PD20	ΔFres (%) PD20	ΔAX (%) PD20
ΔFVC (%) PD20	0.15	-0.17	0.00	-0.07
ΔFEV1 (%) PD20	-0.05	-0.32	-0.09	-0.28
ΔFEF50 (%) PD20	-0.10	-0.25	-0.20	-0.30
ΔRtot (%) PD20	0.34	0.13	0.28	0.35
ΔSRtot (%) PD20	0.34	0.29	0.36	0.44*
ΔSGtot (%) PD20	-0.42*	-0.22	-0.33	-0.43*

Correlations before PD20-histmine. Marked (*) correlations are significant at $p < 0.05$;
N = 31 (Casewise deletion of missing data).
ΔR5 – interval of total resistance; ΔX5 – interval of reactance; ΔFres – interval of resonant frequency; ΔFVC – interval of forced expiratory volume; ΔFEV1 – interval of forced expiratory flow 1; ΔFEF50 – interval of forced expiratory flow 50; ΔRtot – interval of total specific resistance; ΔSRtot – interval of total specific resistance; ΔSGtot – interval of total specific conductance.

Table 4

Pearson's correlation coefficient between changes after histamine bronchoprovocation dose 20 (PD20) [impulse oscillometry (PD20-IOS) vs. spirometry, plethysmography]

Variable	ΔR5 (%) PD20	ΔX5 (%) PD20	ΔFres (%) PD20	ΔAX (%) PD20
ΔFVC (%) PD20	-0.02	-0.27	-0.05	-0.18
ΔFEV1 (%) PD20	-0.05	-0.21	0.10	-0.06
ΔFEF50 (%) PD20	-0.33	-0.27	-0.23	-0.30
ΔRaw (%) PD20	0.74*	0.60*	0.82*	0.88*
ΔSRaw (%) PD20	0.67*	0.53*	0.77*	0.83*
ΔSGaw (%) PD20	-0.40*	-0.45*	-0.40*	-0.47*

Correlations before PD20-histmin. Marked (*) correlations are significant at $p < 0.05$;
N = 31 (Casewise deletion of missing data).
ΔFVC – interval of forced expiratory volume; ΔFEV1 – interval of forced expiratory flow;
ΔFEF50 – interval of forced expiratory flow 50%; ΔRaw – interval of endobronchial resistance;
ΔSraw – interval of specific resistance; ΔSgaw – interval of specific conductance;
ΔR5 – interval of total resistance; ΔX5 – interval of reactance; ΔFres – interval of resonant frequency; ΔAX – interval of Goldman index.

because it has a high variability. Resistance at 5 Hz and Fres are carriers of the interpretation of findings in BPT. Kohlhaufl et al. [28] conducted a trial with methacholine test among healthy nonsmokers and asymptomatic smokers and proved a 3 times higher value of reactance as compared with FEV1 of asymptomatic smokers, which was probably the consequence of the existence of subclinical bronchiolitis [29]. Short et al. [29] compared IOS and spirometry at challange test and bronchodilatory test in the patients who had used beta blocker proparnolol before and two hours after inhalation of histamine and salbutamol. The values of changes of IOS parameters, R5, R5-20, AX, and Fres were higher than spirometry changes, especially R5 and Fres with the mean values of 30.8% and 39.4%, respectively [29].

Frequency changes R5 and Fres before PD20 in the class intervals over 30% of values were present in 20% of the subjects, which means that every fifth BPT can be estimated as positive step before PD20. Frequency changes after PD20 for R5 in 60% of subjects, and for Fres in 80% of respondents in class intervals over 30% of values were most pronounced, making this method sensitive but not completely specific in assessing BPT.

A correlation between IOS and standard methods before PD20 is not significant; this method gives the possibility of different interpretation. Good correlation of impulse oscillometry with bodyplethysmography complements these two methods in the assessment of BPT. Poor connections to spirometry as the gold standard for estimating pulmonary function shows that impulse oscillometry may provide new information in the assessment of BHR. Mansura et al. [30] analyzed the relationship IOS in 20 patients with stable asthma after BPT with methacholine with asthma symptom score, IOS and spirometry. They proved a significant correlation between the score and IOS, but not with spirometry. Hnatiuk et al. [31] compared parameters of IOS (Z-impedance, R5, Fres, PR-periphery resistance) in 48 subjects and spirometry (FEV1) according to increasing doses of methacholine (0.025, 0.25, 2.5, 10, 25 mg/mL). The changes in impedance correlated significantly with changes of FEV1 for all methacholine doses.

Conclusion

This paper demonstrates a sufficient sensitivity of impulse oscillometry to detect the changes in the airways, so it may play a complementary role in the diagnosis of asthma in male military recruits. The value of step parameter changes before PD20 suggests that IOS is sensitive in the detection of BHR.

R E F E R E N C E S

1. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. 2014. Available from: http://www.ginasthma.org
2. *Freed R, Anderson SD, Wyndham J*. The use of bronchial provocation testing for identifying asthma – a review of the problems for occupational assessment and a proposal for a new direction. ADF Health 2002; 3: 77−85.
3. *Niggemann B, Illi S, Madloch C, Völkel K, Lau S, Bergmann R*, et al. Histamine challenges discriminate between symptomatic and asymptomatic children. Eur Respir J 2001; 17(2): 246−53.
4. *Major-Zoričič Z*. Bronchial hyperresponsivnes and nonspecific bronchoprovocational tests. Saopštenja 1991; 29(3−4): 213−21. (Serbian)
5. *Cropp GJ, Bernstein IL, Boushey HA, Hyde RW, Rosenthal RR, Spector SL*, et al. Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. ATS News 1980; spring: 11−9.
6. *Koruga D*. Impulse oscillometry as a new exploratory method in medicine. Pneumon 2005; 42: 75−9. (Serbian)
7. *Smith H, Reinhold P, Goldman M*. Forced oscillation technique and impulse oscillometry. Eur Respir J 2005; 31(5): 72−105.
8. *Bickel S, Popler J, Lesnick B, Eid N*. Impulse Oscillometry Interpretation and Practical Applications. Chest 2014; 146(3): 841−7.
9. *Komarow HD, Myles IA, Uzaman A, Metcalfe DD*. Impulse oscillometry in the evaluation of diseases of the airways in children. Ann Allergy Asthma Immunol 2011; 106(3): 191−9.
10. *Tomalak W, Czajkowska M, Radliński J*. Application of impulse oscillometry in respiratory system evaluation in elderly patients. Pneumonol Alergol Pol 2014; 82(4): 330−5.
11. *Mauer M, Cummings KR*. Impulse oscillometry and respiratory symptoms in World Trade Center responders, 6 years post-9/11. Lung 2010; 188(2): 107−13.
12. *Kuhnle GE, Brandt T, Roth U, Goetz AE, Smith HJ, Peter K*. Measurement of respiratory impedance by impulse oscillometry: Effects of endotracheal tubes. Res Exp Med (Berl) 2000; 200(1): 17−26.
13. *Komarow HD, Young M, Nelson C, Metcalfe DD*. Vocal cord dysfunction as demonstrated by impulse oscillometry. J Allergy Clin Immunol Practice 2013; 1(4): 387−93.
14. *Kahan ES, Martin UJ, Spungen S, Ciccolella D, Criner GJ*. Chronic cough and dyspnea in ice hockey players after an acute exposure to combustion products of a faulty ice resurfacer. Lung 2007; 185(1): 47−54.
15. *Becker S, Reinhold P, Smith HJ, Reiner G*. Relationship between clinical signs and results of impulse oscillometry in pigs originating from the field. Res Vet Sci 2015; 98: 106−11.
16. *Bailly C, Crenesse D, Albertini M*. Evaluation of impulse oscillometry during bronchial challenge testing in children. Pediatr Pulmonol 2011; 46(12): 1209−14.
17. *Koruga D, Plavec G, Ćirić B, Tomic Z, Bošković K*. Using impulse oscillometry in evaluation bronchodilatation respons in patients with chronic cough. Eur Respir J 2008; 32(Suppl 52): 625.
18. *Koruga D, Tomic J, Jovicevic J, Plavec G, Perin B, Ćiric* B, at al. The utility of the impulse oscillometry system (IOS) in assessing bronchodilator responsiveness in patients with asthma and allergic rhinitis. Eur Respir J 2010; 36(Suppl 54): 4634.
19. *Koruga D, Plavec G, Perin B, Ćirić B, Letic V*. Impulse oscillometry in the evaluation of exercise-induced bronchoconstriction in the male army recruits. Eur Respir J 2009; 34(Suppl 53): 393.
20. *Sterk PJ, Fabbri LM, Quanjer PH, Cockroft DW, O'byrne PM, Anderson SD,* at al. Airway responsivvness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Eur Respir J 1993; 6(Suppl 16): 53−83.
21. *Cocroft DW, Berscheid BA*. Standardization of inhalation provocation tests. dose vs concentration of histamine. Chest 1982; 82(5): 572−5.

22. *Ninković M, Radojičić M.* Standardization of bronchial provocation tests using histamine. Vojnosanit Pregl 1988; 45(5): 350−2. (Serbian)
23. *Koruga D.* Aerosol provocation system (APS) -the latest generation Jaeger's system for bronchial provocation challenge. Pneumon 2006; 43: 77−84. (Serbian)
24. *Miller MR.* Standardisation of spirometry. Eur Respir J 2005; 26(2): 319−38.
25. *Pellegrino R.* Interpretative strategies for lung function tests. Eur Respir J 2005; 26(5): 948−68.
26. *Wanger J.* Standardisation of the measurement of lung volumes. Eur Respir J 2005; 26(3): 511−22.
27. *Koruga D.* Original lung function reports v4.52i for impulse oscillometry, spirometry and body plethysmography as designed in the Jaeger's software. Pneumon 2006; 43: 63−6. (Serbian)
28. *Kohlhaufl M, Brand P, Scheuch G, Schulz H, Haussinger K, Heyder J.* Impulse oscillometry in healthy nonsmokers and asymptomatic smokers: effects of bronchial challenge with methacholine. J Aerosol Med 2001; 14(1): 1−12.
29. *Short PM, Williamson PA, Lipworth BJ.* Sensitivity of impulse oscillometry and spirometry in beta-blocker induced bronchoconstriction and beta-agonist bronchodilatation in asthma. Ann Allergy Asthma Immunol 2012; 109(6): 412−5.
30. *Mansura AH, Manneya S, Ayresb JA.* Methacholine-induced asthma symptoms correlate with impulse oscillometry but not spirometry. Respir Med 2008; 102(1): 42−9.
31. *Hnatiuk OW, Niven AS, Hurwitz HM, Sierra AN.* Use of impulse oscillometry in adult bronchoprovocation testing. Chest 2000; 118(Suppl 4): 198S.

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Diagnostic and pathogenetic significance of apolipoprotein disorders in patients with alcoholic fatty liver

Dijagnostički i patogenetski značaj apolipoproteinskih poremećaja kod bolesnika sa alkoholnom masnom jetrom

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Abstract

Background/Aim. Alcohol is the most common cause of fatty liver. Alcohol metabolism takes place in the liver by alcohol dehydrogenase, to toxic acetaldehyde, with fatty acids accumulation in the liver as a consequence. By daily intake of the amount greater than 80 g/day for men and 20 g for women, there is the risk for developing the alcoholic fatty liver (AFLD). The aim of this study was to determine the profile of atherogenic factors in plasma of patients with AFLD compared to patients with non-alcoholic fatty liver (NAFLD) and determine its diagnostic significance. **Methods.** The study included 74 patients with AFLD who consumed alcoholic beverages daily in large quantities and over 80 g [for men: 3–4 units (U) of alcohol and for women 2–3 U]; the control group consisted of 70 patients with NAFLD verified with ultrasound. A total holesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and apoliporoteins (ApoA1 and ApoB) were determined and the ratios TC/HDL-C, ApoB/ApoA1 and LDL-C/HDL-C were calculated. **Results.** The study included two group: 74 AFLD patients (21% of women and 79% of men), mean age 42.65 ± 9.73 years, who consumed alcoholic beverages daily in the amounts of 80 g, or greter, during the average 2.31 ± 0.96 years and 70 patients with NAFLD (37.5% of women and 63.5% of men) with the average 41.3 ± 4.1 years. There was no significant difference in gender distribution and the average age between the examined groups. Higher values of TG – 9.94 ± 2.94 mmol/L, TC 14.53 ± 2.81 mmol/L, LDL-C 8.57 ± 2.15 mmol/L and ApoB 3.97 ± 0.28 g/L and lower values of HDL-C 0.43 ± 0.11 mmol/L, Apo A1 0.49 ± 0.09 g/L and ApoB/ApoA1 ratio 2.43 ± 1.27 were registered in the AFLD group compared to those registered in the NAFLD group, (TG 8.74 ± 2.54 mmol/L TC 9.87 ± 2.36, LDL-C 6.72 ± 1.98 mmol/L, Apo B 2.38 ± 0.16 g/L, HDL-C 0.78 ± 0.09 mmol/L, Apo A1 0.98 ± 0.04 g/L and ApoB/ApoA1 ratio 7.81±1.42). There were no differences in albumin concentration, international normalized ratio (INR) and values of haemoglobin and haematocrit between the groups. **Conclusion.** Lipids and the ApoB/ApoA1 ratio, besides markers of hepatocelular damage, can serve as a diagnostic criteria for the presence of AFLD, and as a better indicator of atherogenic risk.

Key words:
liver disease, alcoholic; non-alcoholic fatty liver disease; risk factors; apolipoproteins a; apolipoproteins b; lipids; diagnosis; alcohol drinking.

Apstrakt

Uvod/Cilj. Alkohol je najčešći uzrok masne jetre. Metabolizam alkohola odvija se u jetri posredstvom alkoholne dehidrogenaze do toksičnog acetaldehida sa posledičnom akumulacijom masnih kiselina u jetri. Svakodnevnim unosom količina većih od 80 g/dan za muškarce, odnosno 20 g za žene, postoji rizik od razvoja alkoholne masne jetre. Cilj rada bio je da se utvrdi profil aterogenih faktora u plazmi bolesnika sa alkoholnom masnom jetrom (AFLD) u odnosu na bolesnike sa nealkoholnom masnom jetrom (NAFLD) i odredi njegov dijagnostički značaj. **Metode.** Studijom je bilo obuhvaćeno 74 bolesnika sa AFLD koji su konzumirali razno alkoholno piće u dnevnoj količini 80 g i više [za muškarce 3–4 jedinice (U) alkhola dnevno, a za žene 2–3 U]; kontrolnu grupu činilo je 70 bolesnika sa ultrazvučno potvrđenom NAFLD. Praćeni su ukupni holesterol (TC), trigliceridi (TG), lipoproteini velike gustine (HDL-C), lipoproteini male gustine (LDL-C) i apolipoproteini (ApoA1 i ApoB) i izračunavan je odnos TC/HDL-C, ApoB/ApoA1 i LDL-C/HDL-C. **Rezultati.** Studijom su bile obuhvaćene: grupe: I – 74 bolesnika (21% žena i 79% muškaraca) sa AFLD, prosečne starosti 42,65 ± 9,73 godina, koji su svakodnevno konzumirali alkoholne napitke u količini većoj od 80 g, tokom prosečno 2,31 ± 0,96 godina, i grupa II – 70 bolesnika sa NAFLD

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(37% žena i 63% muškaraca) prosečne starosti 41,3 ± 4,1 godina. Nije registrovana značajnija razlika u starosti i polu između ispitivanih grupa. Registrovane su značajno više vrednosti TG 9,94 ± 2,94 mmol/L, TC 14,53 ± 2,81 mmol/L, LDL-C 8,57 ± 2,15 mmol/L i Apo B 3,97 ± 0.28 g/L a niže vrednosti HDL-C 0,43 ± 0,11 mmol/L, Apo A1 0,49 ± 0,09 g/L i odnosa ApoB/ApoA1 2,43 ± 1,27, u grupi AFLD u odnosu na grupu NAFLD, (TG 8,74 ± 2,54 mmol/L, TC 9,87 ± 2,36 mmol/L, LDL-C 6,72 ± 1,98 mmol/L, Apo B 2,38 ± 0,16 g/L, HDL-C 0,78 ± 0,09 mmol/L, Apo A1 0,98 ± 0.04 g/L i ApoB/ApoA1 7,81 ± 1,42). Nije nađena značajna razlika u koncentraciji albumina, vrednostima *international normalized ratio* (INR), hemoglobina i hemotokrita između grupa. **Zaključak.** Vrednosti lipidnih frakcija i odnosa ApoB/ApoA1 uz markere hepatocelularnog oštećenja, mogu poslužiti kao dijagnostički kriterijum prisustva AFLD i biti dobar pokazatelj aterogenog rizika.

Ključne reči:
jetra, bolesti izazvane alkoholom; jetra, masna, infiltracija, nealkoholna; faktori rizika; apolipoproteini a; apolipoproteini b; lipidi; dijagnoza; alkohol, pijenje.

Introduction

Alcohol is the most common cause of liver disease in 40–80% of cases. The most common form of alcoholic liver disease is the fatty liver, and the most difficult one, cirrhosis of the liver. Cirrhosis is the 9th cause of death, and in the population aged 45–64 years, it is the 6th one [1–3]. The amount and length of alcohol consumption are related to the manifestation of alcoholic liver disease. The risk of alcoholism is higher with the transition of the threshold of 80 g of alcohol a day for men, and 20 g for women.

A diet deficient in protein and antioxidant vitamins, increases the hepatotoxicity of ethanol. Hepatotoxic effects of ethanol are more noticeable with an increase in intake of unsaturated fatty acids. Ethanol increases the absorption of iron from the intestine and increases its disposal in the liver [4–9].

The clinical picture of alcoholic liver disease varies from asymptomatic disease, fatty liver, alcoholic hepatitis, to heavy cirrhosis of liver with complications [10–12]. The overlapping of more than one form of alcoholic liver disease in the same patient is often present. Alcoholic fatty liver disease (AFLD) is often not recognized. The classic clinical picture is presented from asymptomatic state to the manifestation of weakness, fatigue, nausea, and rarely anorexia, vomiting and diarrhea. In laboratory the cytolysis of hepatocytes and retention of bilirubin are present, and rarely reduced synthetic liver function.

The diagnosis is based on properly taken history, including the data on alcoholism, elevated values of aspartate transaminase (AST) and alanine transaminase (ALT) [13]. Alkaline phosphatase (ALP) may be slightly elevated in contrast to cirrhosis, which is up to 4 times higher. Gamma glutamyl transferase (GGT) is induced by alcohol, and quickly returns to normal after stopping the use of alcohol [14–16]. Hyperbilirubinemia and prolonged prothrombin time is more common in more severe forms of alcoholic liver disease. Hyperuricemia and dyslipidemia, follow the chronic alcoholism [4–6]. More severe forms of alcoholic liver disease are compained by leukocytosis with neutrophilia and anemic syndrome as well as hypoalbuminemia with hypergammaglobulinemia. In chronic consumption of alcohol the values of IgA are elevated [17–21]. In the diagnosis of alcoholic liver disease, the biopsy of liver occupies an important place [22–24].

Non-alcoholic fatty liver disease (NAFLD) is a disease of the liver which has histologic features of alcoholic liver disease, and wherein the persons do not consume alcohol. The development of NAFLD can be influenced by drugs or toxins. It can be hereditary and acquired disorders of metabolism. The clinical picture of NAFLD is rarely characterized by nonspecific signs such as fatigue, malaise, mild pain under the right rib arch. This is mostly asymptomatic disease. In the clinical findings of 75% of patients hepatomegaly is registered, and in 25% splenomegaly. It rarely goes into the fulminant form with rapid development of cirrhosis and death.

The diagnosis is set by the history of significant absence of alcohol consumption. In laboratory the moderate rise in aminotransferases, and AST/ALT ratio is less than 1. The disorders of synthetic function of the liver and retention of bilirubin are rare. In a number of patients hyperglycemia, hypertriglyceridemia and hypercholesterolemia are present. Liver biopsy is the gold standard for diagnosis.

The aim of this study was to evaluate the profile of atherogenic factors in plasma in patients with AFLD and patients with NAFLD and determine its diagnostic significance.

Methods

The study included 74 patients with AFLD who consumed alcoholic beverages daily in large quantities and over 80 g; for men it is the amount of 3–4 units (U) of alcohol, and for women 2–3 (one alcoholic U corresponds to approximately single spirit, or 0.5 L of beer or 2 dL wine spritzer or 4 oz) [6]. The control group consisted of 70 patients with verified NAFLD. The diagnosis of NAFLD is set by ultrasonography of abdomen. All the patients were submitted to laboratory processing, AST and ALT, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and apolipoproteins (Apo) – ApoA1 and ApoB and blood cells count. The ratios of TC/HDL-C, ApoB/ApoA1 and LDL-C/HDL-C were calculated.

The diagnosis of NAFLD was carried out with the ultrasound apparatus Siemens X 300 in a supine position with the World Health Organization (WHO) diagnostic criteria applied from 2005. On that occasion, the so-called phenomena was compared: brightness, deep beam attenuation, liver-kidney contrast, gallbladder wall definition.

By blood tests, from the studies were excluded the patients with viral hepatitis and autoimmune disorders, and by ultrasound examination, the patients with tumor processes and obstruction of the biliary tree.

In this paper, we used the standard descriptive methods, mean values, standard deviation and percentage distribution, and the data between the groups were analyzed by appropriate statistical tests depending on the type and distribution of features (Man Whitney U test, Student's *t*-test, χ^2 test). The significance level of $p < 0.05$ was taken as significant. Data are presented as tabulated.

Results

The study included 144 patients, of whom 37.5% were women and 62.5% men, the mean age of 42.05 ± 8.56 years. The patients were divided into two groups, with AFLD and NAFLD.

The first group included 74 patients with AFLD, aged 22–67 years, the average age of 42.65 ± 9.73 years. They consumed alcoholic drinks in greater quantity than 80 g for 1 to 4 years on the average 2.31 ± 0.96 years. Among the respondents, there was 21% of women and 79% of men. In the second group of patients, there were 70 subjects with NAFLD, aged 24–65 years, mean age 41.31 ± 4.1 years, who were not consuming alcoholic beverages. Among them, there was 37.5% of women and 63.5% of men. There was no significant difference in gender distribution and the average age between the examined groups (Table 1).

The average values of AST, ALT, GGT and AST/ALT ratio in the AFLD group were significantly higher than in the NAFLD group ($p < 0.01$) prospectively. There were no differences in albumin concentration, international normalized ratio (INR) and parameters of anemia between the groups. The number of leukocytes was significantly higher and platelets lower in the AFLD group ($p < 0.05$) (Table 2).

Table 1

General characteristics of the studied group of patients

Fatty liver groups	Women / men	Age (years)	Length of alcohol consumption (years)
	n (%)	x̄ ± SD	x̄ ± SD
Alcoholic	21/79 (28/46)	42.65 ± 9.73	2.31 ± 0.96
Non-alcoholic	37.5/63.5 (26/44)	41.31 ± 4.1	-
Total	54/90 (37.5/62.5)	42.05 ± 4.2	-

x̄ – mean values; SD – standard deviation.

Table 2

Biochemical and haemathological parameters in hepatocellular injury

Parameters	Alcoholic fatty liver (x̄ ± SD)	Non-alcoholic fatty liver (x̄ ± SD)
Liver function tests		
AST (U/L)	61.26 ± 8.45	45.37 ± 6.23**
ALT (U/L)	73.69 ± 12.69	59.76 ± 10.31**
AST/ALT	0.83	0.76**
GGT (U/L)	96.7 ± 12.42	83.2 ± 9.56**
Albuminical (g/L)	41.04 ± 5.54	41.03 ± 8.43
INR	1.3 ± 0.09	1.3 ± 0.08
Haemathological parameters		
leukocytes ($\times 10^9$/L)	8.43 ± 0.88	7.96 ± 0.79*
Hb (g/L)	144.4 ± 12.49	145.2 ± 12.31
Hct (%)	42.39 ± 3.17	44.27 ± 3.73
PLT ($\times 10^9$/L)	207.12 ± 39.11	221.09 ± 36.34*
Atherogenic indicators		
TC (mmol/L)	14.53 ± 2.81	9.87 ± 2.36**
LDL-C (mmol/L)	8.57 ± 2.15	6.72 ± 1.98**
HDL-C (mmol/L)	0.43 ± 0.11	0.78 ± 0.09**
TG (mmol/L)	9.94 ± 2.94	8.74 ± 2.54 **
TC/HDL-C	32.61 ± 9.21	12.65 ± 7.25**
LDL-C/HDL-C	17.39 ± 5.01	8.62 ± 3.25**
ApoB (g/L)	3.97 ± 0.28	2.38 ± 0.16**
ApoA1 (g/L)	0.49 ± 0.09l	0.98 ± 0.04**

Hb – haemoglobin; Hct – haematocrit; PLT – platelets; INR – international normalized ratio; GGT – gamma glutamyl tranferase; AST – aspartate aminotransferase; ALT – alanine aminotransferase; TC – total cholesterol; TG – triglycerides; ApoA1 – apolipoprotein A1; ApoB – apolipoprotein B; LDL-C low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol.

x̄ – mean value; SD – standard deviation; *$p < 0.05$, **$p < 0.01$ *vs* alcoholic fatty liver.

All the examined patients had highly elevated TC (more than 6.2 mmol/L) and TG (more than 5.65 mmol/L) as well as highly reduced HDL-C (less than 1 mmol/L) in both AFLD and NAFLD group. All the patients with AFLD had values of LDL-C greater than 4.9 mmol/L while this was not the case in the NAFLD group.

The index TC/HDL-C, was elevated and at high-risk (more than 4.5) with the average value of 32.61 ± 9.21 in the patients with AFLD and 12.65 ± 7.25 in the NAFLD group. The ApoB/ApoA1 index was calculated, and all the values in both groups were in over 1.1 which also represents high risk. The index LDL-C/HDL-C was greater than 3.5 which represents a very high atherogenic risk in all patients.

There were significant differences in lipid parameters and apolipoproteins between the groups. Higher values of TG, TC, LDL-C, ApoB and ApoB/ApoA1 ratio, and lower values of HDL-C, ApoA1 were registered in the AFLD compared to the NAFLD group (Table 2).

Discussion

The examined groups of patients with AFLD and NAFLD were predominantly male with similar average age. During the relatively short period of alchocol consumption in AFLD there was a manifestation of the most lenient alcoholic liver damage, fatty liver. All the patients had elevated transaminases, with ALT predominance, so the AST/ALT was 0.83. The values of GGT were also elevated. In the NAFLD group of not consumers of alcoholic beverages, the transaminases and GGT were increased, with the predominance of ALT. The AST/ALT ratio was lower (0.76) compared with the patients with AFLD (Tables 1 and 2).

Laboratory analysis verified the elevation of the values of TC, TG and LDL-C, as atherogenic factors, and decreased values of antiatherogenic HDL-C. The lipid disorders were more prominent in the AFLD group than in the NAFLD group (Table 3).

Alcohol in the body is the subject of oxidation, mainly in the liver. Ethanol is metabolized in acetate by using three enzymatic systems: alcohol dehydrogenase (ADH), the microsomal oxidation system (MEOS) and catalase. Ethanol is oxidized, 80–85%, to highly toxic acetaldehyde which damages the cell membrane, leading to cell necrosis. Ethanol oxidation produces the accumulation of fatty acid and triglycerides in the liver and increased lipoprotein synthesis. The increased concentration of NADH changes the redox potential of hepatocytes, leading to the inhibition of protein synthesis, rise of lactate and urate levels in serum. The MEOS is responsible for the metabolism of 10–15% of ethanol. Thus, the consumption of oxygen and the production of acetaldehyde is increased, and also the lipid peroxidation. Catalase system is poorly active in the metabolism of alcohol [4–7].

The values of ApoA1 and ApoB were also determined. Among the patients with AFLD, ApoA1, ApoB/ApoA1 ratio and HDL-C were lower compared to the NAFLD group. ApoA1 is a component of an antiatherogenic lipoprotein and was decreased in AFLD (Table 3). Low values of ApoA1 created conditions for the development of atherogenic effect. ApoB as atherogenic component was extremely increased in AFLD. Due to the reduced ApoA1 and increased ApoB, in patients with chronic consumption of ethanol and developed AFLD and NAFLD, atherogenic effect in plasma was created [4, 20, 21].

Apo B is the primary Apo of chylomicrons and LDL, and is responsible for transporting of TC to tissues [5, 8]. ApoB in particles of LDL is a ligand for the LDL receptors of the cells, and "unlocks" cells, and transport TC to them. By an unknown mechanism of high value ApoB lead to the formation of plaques in blood vessels and the development of atherosclerosis. Thus, the determination of ApoB is a better and more significant indicator of atherosclerosis risk than analysis of LDL and total TC. As in the patients with AFLD there is a significant elevation of ApoB, there is an increased risk of plaques formation in blood vessels, and so, consequently, for the development of atherosclerosis.

Apo A1 is a major component of plasma HDL-C. It leads to the so-called fat-efflux from tissues to the liver [14]. Thus mobilized fat is than excreted from the liver. ApoA1 is a cofactor of lecithin cholesterol transferase (LCAT), important for the synthesis of TC esters in the plasma. ApoA1 is an ingredient of prostacyclin (PGI2), responsible for the realization of antiagregational effects.

The study Incremental Decrease and Events through Aggressive Lipid Lowering (IDEAL) [25], and INTERHEART study [26], emphasize that the determination of the ratio ApoB/ApoA1 is a significant prognostic factor of atherogenic effects. Individual monitoring of TC, LDL-C or ApoB, as atherogenic factors, and HDL-C, and Apo-A1, as antiatherogenic factors, as well as determining the ratios TC/HDL-C or LDL-C/HDL-C is less significant as compared to ApoB/ApoA1 ratio.

Determining the relationship between ApoB/ApoA1 proved to be the most important and most coherent marker for the existence of atherogenic plasma.

Conclusion

Determination of TC, TG, LDL-C, HDL-C, ApoA1, ApoB1 and ratios ApoB/ApoA1, LDL-C/HDL-C, TC/HDL-C is an important parameter of tracking of atherogenic factors in plasma. Both AFLD and NAFLD lead to increase of ApoB, an indicator of atherogenic status of plasma, and increased risk for the formation of plaques in blood vessels, including atherosclerosis. The values of TC, TG, LDL-C, HDL-C, LDL-C/HDL-C and TC/HDL-C ratios showed great variation from mild to very elevated. Determining the value of the ApoB/Apo A1 ratio is less variable, more coherent parameter for tracking of atherogenic changes in plasma. The lipids and ApoB/ApoA1 ratio besides markers of hepatocelular damage can serve as a diagnostic criteria for the presence of AFLD, and as a better indicator of atherogenic risk.

R E F E R E N C E S

1. *Mandayam S, Jamal M, Morgan TR.* Epidemiology of Alcoholic Liver Disease. Semin Liver Dis 2004; 24(3): 217−32.
2. Alcohol use and alcohol use disorders in the United States: Main findings from the 2001-2002 National Epidemiologic Survey on Alcohol Use and Related Conditions (NESARC). Bethesda, Md: National Epidemiologic Survey on Alcohol Use and Related Conditions (NESARC); National Institutes of Health (U.S.); National Institute on Alcohol Abuse and Alcoholism (U.S.); CSR. 2006. Available from: http://www.ginasthma.org
3. *Yoon YH, Yi HY.* Surveillance report #75: Liver Cirrhosis Mortality in the United States, 1970-2003. Bethesda, MD: National Institute on Alcohol Abuse and Alcoholism; 2006.
4. *Sougioultzis S, Dalakas E, Hayes PC, Plevris JN.* Alcoholic hepatitis: From pathogenesis to treatment. Curr Med Res Opin 2005; 21(9): 1337−46.
5. *Lieber CS.* New concepts of the pathogenesis of alcoholic liver disease lead to novel treatments. Curr Gastroenterol Rep 2004; 6(1): 60−5.
6. *Kamper-Jorgensen M, Gronbaek M, Tolstrup J, Becker U.* Alcohol and cirrhosis: Dose-response or threshold effect. J Hepatol 2004; 41(1): 25−30.
7. *Pessione F, Ramond M, Peters L, Pham B, Batel P, Rueff B,* et al. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. Liver Int 2003; 23(1): 45−53.
8. *Mendez-Sanchez N, Meda-Valdes P, Uribe M.* Alcoholic liver disease. An update. Ann Hepatol 2005; 4(1): 32−42.
9. *Leevy CM, Moroianu SA.* Nutritional aspects of alcoholic liver disease. Clin Liver Dis 2005; 9(1): 67−81.
10. *Lefkowitch JH.* Morphology of alcoholic liver disease. Clin Liver Dis 2005; 9(1): 37−53.
11. *Barrio E, Tome S, Rodriguez I, Gude F, Sanchez-Leira J, Perez-Becerra E,* et al. Liver Disease in Heavy Drinkers With and Without Alcohol Withdrawal Syndrome. Alcohol Clin Exp Res 2004; 28(1): 131−6.
12. *Aertgeerts B, Buntinx F, Kester A.* The value of the CAGE in screening for alcohol abuse and alcohol dependence in general clinical populations: A diagnostic meta-analysis. J Clin Epidemiol 2004; 57(1): 30−9.
13. *Nyblom H.* High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol Alcohol 2004; 39(4): 336−9.
14. *Hannuksela ML, Liisanantti MK, Nissinen AE, Savolainen MJ.* Biochemical markers of alcoholism. Clin Chem Lab Med 2007; 45(8): 953−61.
15. *Anttila P, Jarvi K, Latvala J, Romppanen J, Punnonen K, Niemela O.* Biomarkers of alcohol consumption in patients classified according to the degree of liver disease severity. Scand J Clin Lab Invest 2005; 65(2): 141−51.
16. *Niemela O.* Biomarkers in alcoholism. Clin Chim Acta 2007; 377(1−2): 39−49.
17. *Shiffman RN, Shekelle P, Overhage JM, Slutsky J, Grimshaw J, Deshpande AM.* Standardized reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. Ann Intern Med 2003; 139(6): 493−8.
18. *Levitsky J, Mailliard ME.* Diagnosis and Therapy of Alcoholic Liver Disease. Semin Liver Dis 2004; 24(3): 233−47.
19. *Aalto M, Seppä K.* Use of laboratory markers and the audit questionnaire by primary care physicians to detect alcohol abuse by patients. Alcohol Alcohol 2005; 40(6): 520−3.
20. *Alte D, Luedemann J, Rose HJ, John U.* Laboratory markers carbohydrate-deficient transferrin, gamma-glutamyltransferase, and mean corpuscular volume are not useful as screening tools for high-risk drinking in the general population: results from the Study of Health in Pomerania (SHIP). Alcohol Clin Exp Res 2004; 28(6): 931−40.
21. *Chen J.* Combining carbohydrate-deficient transferrin and gamma-glutamyltransferase to increase diagnostic accuracy for problem drinking. Alcohol Alcohol 2003; 38(6): 574−82.
22. *Trabut JB, Plat A, Thepot V, Fontaine H, Vallet-Pichard A, Nalpas B,* et al. Influence of Liver Biopsy on Abstinence in Alcohol-Dependent Patients. Alcohol Alcohol 2008; 43(5): 559−63.
23. Helping Patients Who Drink Too Much: A Clinician's Guide. Bethesda, MD: National Institute of Health, National Institute on Alcohol Abuse and Alcoholism; 2005.
24. *Daniel JR, Helen HH.* Disorders of Lipoprotein metabolism. In*: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J,* editors. Harrison's Principles of Internal Medicine. 18th ed. New York: McGrawHill Medical Publisher Division; 2012. p. 3145−61.
25. *Rosengren A, Hawken S, Ounpuu S, Sliwa K, Zubaid M, Almahmeed WA,* et al. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364(9438): 953−62.
26. *Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I,* et al. Incremental Desrease and Events through Aggressive Lipid Lowering (IDEAL). Am J Cardiol 2004; 94(6): 720−4.

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Effects of bruxism on the maximum bite force

Uticaj bruksizma na maksimalnu zagrižajnu silu

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Abstract

Background/Aim. Bruxism is a parafunctional activity of the masticatory system, which is characterized by clenching or grinding of teeth. The purpose of this study was to determine whether the presence of bruxism has impact on maximum bite force, with particular reference to the potential impact of gender on bite force values. **Methods**. This study included two groups of subjects: without and with bruxism. The presence of bruxism in the subjects was registered using a specific clinical questionnaire on bruxism and physical examination. The subjects from both groups were submitted to the procedure of measuring the maximum bite pressure and occlusal contact area using a single-sheet pressure-sensitive films (Fuji Prescale MS and HS Film). Maximal bite force was obtained by multiplying maximal bite pressure and occlusal contact area values. **Results**. The average values of maximal bite force were significantly higher in the subjects with bruxism compared to those without bruxism ($p < 0.001$). Occlusal contact area was significantly higher in the subjects suffering from bruxism ($p < 0.001$), while the maximal bite pressure values did not show a significant difference between the studied groups ($p > 0.01$). Maximal bite force was significantly higher in the males compared to the females in all segments of the research. **Conclusion**. The presence of bruxism influences the increase in the maximum bite force as shown in this study. Gender is a significant determinant of bite force. Registration of maximum bite force can be used in diagnosing and analysing pathophysiological events during bruxism.

Key words:
bruxism; bite force; dental occlusion; sex; male; female.

Apstrakt

Uvod/Cilj. Bruksizam je parafunkcionalna aktivnost mastikatornog sistema, koja se karakteriše stezanjem ili struganjem zubima. Cilj ove studije bio je da se utvrdi da li prisustvo bruksizma ima uticaja na maksimalnu zagrižajnu silu, sa posebnim osvrtom na potencijalni uticaj pola na vrednosti zagrižajne sile. **Metode**. Ova studija je obuhvatila dve grupe ispitanika: ispitanike sa bruksizmom i bez bruksizma. Prisustvo bruksizma kod ispitanika je registrovano upotrebom specifičnog kliničkog upitnika za bruksizam i kliničkim pregledom. Ispitanici obe grupe bili su podvrgnuti postupku merenja maksimalnog zagrižajnog pritiska i okluzalne kontaktne površine upotrebom jednoslojnih filmova osetjivih na pritisak (Fuji Prescale MS i HS Film). Maksimalna zagrižajna sila dobijena je množenjem vrednosti maksimalnog zagrižajnog pritiska i okluzalne kontaktne površine. **Rezultati**. Prosečne vrednosti maksimalne zagrižajne sile bile su značajno veće kod ispitanika sa bruksizmom nego kod ispitanika bez bruksizma ($p < 0,001$). Okluzalna kontaktna površina bila je značajno veća kod ispitanika koji pate od bruksizma ($p < 0,001$), dok vrednosti maksimalnog zagrižajnog pritiska nisu pokazale značajnu razliku između ispitivanih grupa ($p < 0,01$). Maksimalna zagrižajna sila bila je veća kod muških ispitanika nego kod ženskih ispitanika, u svim segmentima istraživanja. **Zaključak**. Prisustvo bruksizma uticalo je na povećanje maksimalne zagrižajne sile u ovoj studiji. Pol je bio značajna determinata zagrižajne sile. Registracija maksimalne zagrižajne sile može se koristiti za dijagnozu i analizu patofizioloških događaja tokom bruksizma.

Ključne reči:
bruksizam; zagrižajna sila; zubi, okluzija; pol; muškarci; žene.

Introduction

Bruxism is a parafunctional activity of the masticatory system, which is characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible [1]. It may happen while awake (awake bruxism) or while sleeping (sleep bruxism). Bruxism during daytime is commonly a semi-voluntary clenching activity or diurnal bruxism. Awake bruxism can be associated with life stress caused by familial responsibility or work pressure. Sleep bruxism is an oromandibular behavior that is defined as a stereotyped movement disorder occurring during sleep and characterized by tooth grinding and/or clenching [2].

Bruxism is a multifactorial disorder. Bruxism and grinding have been associated with peripheral factors, such as to-

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oth interference in dental occlusion, psychosocial influences, such as stress or anxiety [3], and central or pathophysiological causes involving brain neurotransmitters or basal ganglia [4]. Manfredini et al. [5] indicate that occlusal factors do not seem to have any significant role in the development of bruxism. Depression, increased level of hostility [6] and stress sensitivity [7] distinguish a "bruxer" from a healthy individual. However, factors like smoking, alcohol, drugs, diseases, and trauma may also be involved in the bruxism etiology [8].

Factors that may indicate the presence of bruxism include physical symptoms and changes in hard and soft oral tissues. The physical symptoms of bruxism may include: headache, facial myalgia (muscle pain) and temporomandibular joint (TMJ) discomfort. The most common oral symptoms include: abnormal tooth wear (attrition on occlusal or incisal surfaces), fracture of the teeth and excessive tooth mobility.

In "bruxers", the distribution of muscular force to the teeth and surrounding tissues may result in tooth wear and orofacial pain, as well as hyperactivity and hypertrophy of the masticatory muscles, especially the masseter muscle. In view of the fact that muscles are the main bite force generators, the changes in their function may be reflected in the maximum bite force (MBF) value. MBF is a result of the masticatory muscle activity, which is regulated by the central nervous system receptors and orofacial structures (muscle spindles, proprioceptors, mechanoreceptors). Previous studies report that MBF may be influenced by gender, craniofacial morphology, periodontal sensitivity, dental occlusal status and signs and symptoms of temporomandibular disorders [9–11].

Reports of certain studies on the effects of bruxism on MBF appear to be contradictory. Helkimo and Ingervall [12] found that individuals with clenching and grinding habits had higher bite force only on the incisors, but not on the molars. On the other hand, Gibbs et al. [13] found higher bite force values on the posterior region for subjects with bruxism than for the control group. Lyons and Baxendale [14] suggested that the jaw-closing muscles of subjects with bruxism might have benefited from a "training effect" as a result of all this activity, resulting in muscles that are stronger and possibly more resistant to fatigue. Cosme et al. [15] believe that bruxism does not affect MBF, while some of the authors find that MBF is increased in 54.5% of the subjects suffering from bruxism [16]. According to Nunes [17], for some patients pain plays a modulator role in parafunctional activity, decreasing the electromyographic activity of masticatory muscles and MBF.

There seems to be no clear correlation between the MBF and bruxism. In view of the aforementioned, the main purpose of this study was to determine whether bruxism has impact on MBF, assessing the potential gender impact on the MBF values.

Methods

This trial was conducted ensuring the full adherence to the principles of the "Good Clinical Practice (GCP)" which means that the trial included only participants who had given their full informed consent to participate in writing, with a prior access to the full information about the aims and scope of the trial. This trial was conducted with the approval of the Ethics Committee at the Faculty of Medicine, University of Priština/Kosovska Mitrovica.

The trial was conducted on the subjects selected among the students of the Faculty of Medicine in Kosovska Mitrovica and the patients who visited the Prosthodontics Clinic, Dentistry Department, Faculty of Medicine in Kosovska Mitrovica.

The presence/absence of bruxism in subjects were registered using a specific clinical questionnaire on bruxism by Molina et al. [18] and specific physical examinations.

The Molina questionnaire included the following questions: 1) Do you wake up in the morning or during the night to find yourself grinding or clenching? 2) Do you feel fatigue or masticatory muscle pain on awakening? 3) Do you wake up in the morning or during the night with the jaws locked? 4) Do you feel discomfort on the teeth on awakening? 5) Do you have recent history of chronic dislocation of permanent or temporary restorations? 6) Do you have recent history of noises associated with nocturnal teeth grinding as reported by a third person?

Physical examination included observation of attrition on occlusal or incisal surfaces, detectable scars and buccal mucosa changes, changes on the lateral border of the tongue (tongue indentations) and verification of masticatory muscle hypertrophy by means of digital palpation in maximum intercuspation.

Signs and symptoms of temporomandibular disorders (TMD) were recorded by Helkimos clinical functional analysis [19]. This analysis includes the case history (questionnaire relating to the signs and symptoms of TMD), clinical functional analysis of the orofacial system and occlusal analysis.

Group formation

The following exclusion criteria were applied for all participants: more than two missing posterior teeth (excluding third molars); previous orthodontic or prosthodontic treatment; the presence of active phase of periodontal disease; signs and symptoms of TMD or spontaneous orofacial pain; the presence of malocclusion (anterior open bite, unilateral cross bite, class II and III malocclusion according to Angle).

Further criteria for inclusion subjects in the study implied: the intact dental arch (third molars not taken into account); the presence of no more than three fillings; Class I neutro-occlusion according to Angle's classification; age between 18 to 23 years.

The subjects included in the study, in terms of the registered presence/absence of bruxism, were divided into two groups: the study and the control group. The study group consisted of 41 patients with bruxism, while the control group consisted of 48 subjects without bruxism (18–23 years of age).

Registration of maximum bite force

Further research implied registration of maximum bite pressure (MBP), occlusal contact area and calculation of

MBF value in both the control and the experimental (study) group. MBP was registered by means of a single sheet pressure-sensitive sheet (Fuji Prescale, Tokyo), type: MS and HS. MS pressure-sensitive sheet registered pressure within the range of 10–50 megapascal (Mpa), while HS sheet registered the pressure of 50–130 MPa. Fuji Prescale Film technology and its principle of operation is based on indicating applied pressure differences as red color density variations. This feature is enabled by particle size control (PCS) technology based on microcapsule layers designed to respond to different pressures relieving color whose intensity is proportional to the pressure applied.

The MBP registration procedure was conducted in both the study and the control group. The subjects were comfortably seated with the head erect and torso in upright position. Drying provided a relatively dry environment in biting surfaces for placing a horseshoe-shaped pressure sensitive sheet in-between. The subjects were instructed to bite stronger in maximum intercuspation and maintain the bite force the following 10 s (Figure 1 a and b).

The registration procedure was conducted by means of MS and HS pressure sensitive sheet in all the patients, with a 2-minute break between the two registration protocols, to allow for the masticatory muscles to relax. The films applied were further on scanned using a Canon device generating 300 dpi A4 scans. Visual comparison of the occlusal contact color and color intensity scale (0.1 to 1.5) was used for the purpose of defining color density (intensity) for each occlusal contact registered (Figure 2).

Based on the color density, reading of the bite pressure values was carried out for each occlusal contact (Figure 3). The graph shows two curves (A and B).

Occlusal contact area (OCA) was measured by means of Adobe Photoshop 7.0 applied to pressure sensitive sheet

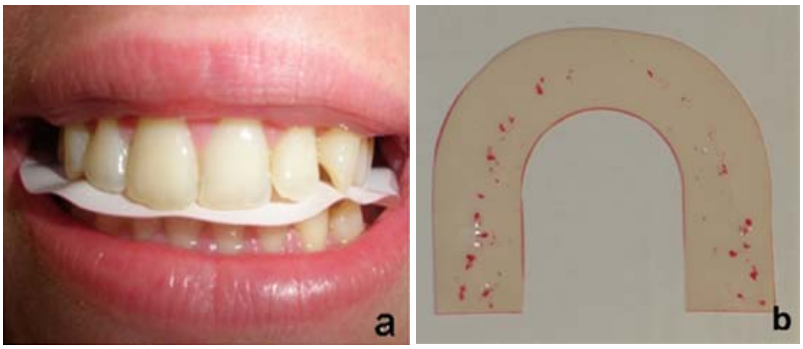

Fig. 1 – Registration of maximum bite pressure (MBP): a) registration procedure; b) registered occlusal contacts on a prescale film.

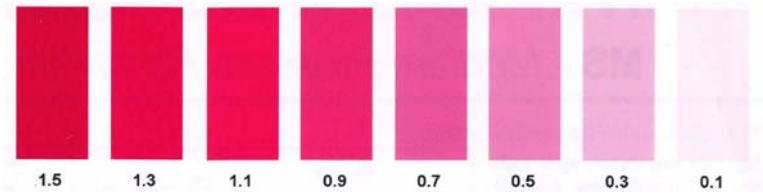

Fig. 2 – Scale for reading color intensity of the registered occlusal contact.

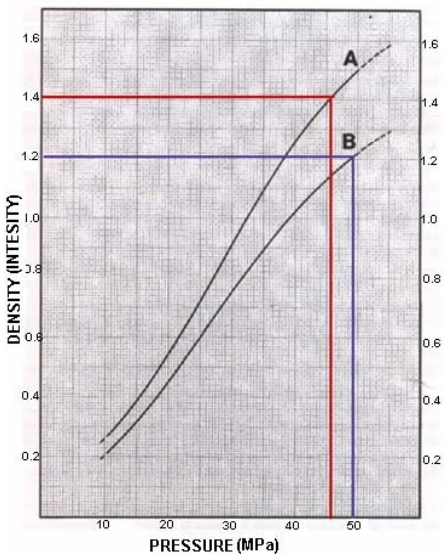

Fig. 3 – Graph for determining values of bite pressure.

scans. Multiplying the values of MBP and OCA, gave the bite force for each occlusal contact observed:

$$F\ (N) = P\ (MPa) \times A\ (mm^2)$$

The sum of all occlusal forces acting in the contact points registered in one patient gave MBF *per* patient.

$$\Sigma\ Fn = F1 + F2 + F3 + ... F_n$$

For the purpose of primary data analysis, methods of descriptive statistics were used, which included measures of central tendency (mean and median), measures of variability (standard deviation) and relative numbers. The influence of bruxism on the MBF value was determined by the Student's *t*-test and the Mann-Whitney Test (Rank Sum Test). Statistical hypotheses were tested at the level of statistical significance of 0.01 and 0.001. For statistical data analysis, a PASW Statistics was used.

Concerning the MBP analysis, values expressed *per* unit area (MBP/mm^2) were used in order to simplify the analysis. Similarly, OCA (mm^2) was analyzed as the sum of the values of each OCA registered in one patient (ОКП = ΣА). However, in calculating the MBF, the values of MBP and contact surface values *per* occlusal contact were used.

Results

Distribution of participants in the study in relation to bruxism and gender is given in Table 1. The first segment of the analysis was conducted in order to test the impact of gender on MBF, which further determined the method of data processing. Thus, comparative analysis of average MBP/mm^2, OCA and MBF values was conducted between the males and females within the control group – patients without bruxism (Table 2). In the male subjects without bruxism, the values of MBF, OCA and MBP/mm^2 were significantly higher than in the female subjects (t = -2975, DF = 54, $p < 0.01$ for MBF; t = -6.825, DF = 54, $p < 0.001$ for OCA; t = -6.944, DF = 54, $p < 0.001$ for MBP/mm^2). Since significant effects of gender on MBP/mm^2, OCA and MBF were found, there was the need to test the values of these parameters comparing separately the male and female participants of both groups (the study group and controls). It was the only way to determine the actual impact of bruxism on the MBF.

In the female subjects with bruxism, the values of MBF and OCA were significantly higher than in the females without bruxism (t = -6.5, DF = 46, $p < 0.001$ and t = -6786, DF = 46, $p < 0.001$, respectively). However, the MBP/mm^2 values did not show any statistically significant difference between the female subjects with and without bruxism (Mann-Whitney test, U = 178.0; p = 0,247) (Table 3).Comparative analysis between the males of both groups showed a statistically significant difference in average values of MBF and OCA (t = -5.440, DF = 27, $p < 0.001$ and t = -4.288, DF=27, $p < 0.001$, respectively). However, in male subjects with bruxism, the MBP/mm^2 values did not show statistically significant difference compared to the males without bruxism (Table 4).

Disscusion

MBF is often analyzed as an indicator of functional status of the masticatory system. Bruxism is one of the parafunctional activities accompanied by rapid contractions of the masseter muscle and development of forces excessively burdening structures of the masticatory system. Harmful effects of bruxism can be seen in non-physiological tooth wear, masticatory muscle hyperactivity and potential development of orofacial system dysfunction. The hypothesis that bruxism is capable to change the bite force by muscle strengthening is still unproven. If the bite force was truly affected by bruxism, its measurement could be an important feature in the diagnosis of such a habit.

Table 1

Distribution of the subjects in relation to bruxism and gender

Gender	Study group, n (%)	Control group, n (%)	Total, n (%)
Females	22 (53.7)	25 (52.1)	47 (52.8)
Males	19 (46.3)	23 (47.9)	42 (47.2)
Total	41 (100.0)	48 (100.0)	89 (100.0)

Study group – subjects with bruxism;
Control group – subjects without bruxism.

Table 2

Comparative analysis of maximum bite pressure (MBP), occlusal contact area (OCA) and maximum bite force (MBF) between the female and male subjects of the control group (without bruxism)

Parameter	Females (n = 25) $\bar{x} \pm SD$	Males (n = 23) $\bar{x} \pm SD$	p*
Number (n)	25	23	
MBP (MPa/mm^2)	36.9 ± 2.50	39.3 ± 3.73	< 0.01
OCA (mm^2)	12.1 ± 3.92	19.5 ± 3.88	< 0.001
MBF (N)	522.60 ± 147.99	793.80 ± 129.78	< 0.001

*** *p* – statistical significance (Student's *t*-test).**

Table 3

Comparative analysis of maximum bite pressure (MBP), occlusal contact area (OCA) and maximum bite force (MBF) between female subjects of both groups

Female subject	Study group (n = 22) $\bar{x} \pm SD$	Control group (n = 25) $\bar{x} \pm SD$	p^*
Number	22	25	
MBP (MPa/mm^2)	37.5 ± 3.62	36.9 ± 2.50	0.247
OCA (mm^2)	20.5 ± 3.54	12.1 ± 3.92	< 0.001
MBF (N)	811.8 ± 27.60	522.6 ± 25.01	< 0.001

***p* – statistical significance at the level < 0.001 for Mann-Whitney *U*-test and < 0.001 for Student's *t*-test;**
Study group – subjects with bruxism;
Control group – subjects without bruxism.

Table 4

Comparative analysis of maximum bite pressure (MBP), occlusal contact area (OCA) and maximum bite force (MBF) between the male subjects of the studied groups

Male subject	Study group (n = 19) $\bar{x} \pm SD$	Control group (n = 23) $\bar{x} \pm SD$	p^*
Number (n)	19	23	
MBP (MPa/mm^2)	42.0 ± 2.83	39.3 ± 3.73	0.079
OCA (mm^2)	25.9 ± 2.77	19.5 ± 3.88	< 0.001
MBF (N)	1,058.4 ± 68.677	793.8 ± 129.78	< 0.001

***p* statistical significance (Student's *t*-test);**
Study group – subjects with bruxism;
Control group – subjects without bruxism.

Our study showed that the average values of MBP/mm^2, OCA and MBF were significantly higher in males compared to females. Some of studies support the results obtained accordingly [20]. Pereira-Cenci et al. [21] and Bonakdarchian et al. [22] believe that greater muscle potential of masticatory muscles in males can be attributed to anatomical gender differences. Bakke [23] points out that masseter muscles of males are type II muscle fibers, which are larger in diameter compared to those in females. Pizolato et al. [24] suggest that hormonal differences between sexes affect the structure of muscle fibers. Estimating contribution of masseter, temporal muscle, and anterior angle of digastric muscle to bite force, Raadsheer et al. [25] demonstrated that masseter thickness significantly correlates with the magnitude of MBF. However, up to 18 years of age, gender does not affect the MBF. Following a post-pubertal period, MBF tends to increase significantly and to a greater extent in men than in women, becoming thus gender-related [26]. According to Olthoff et al. [27], bite force and the number of teeth in occlusion are determining factors in masticatory performance, whereas occlusal contacts determine 10–20% of MBF variation. Ferrario et al. [20] emphasize that dental size is larger in males, making thus the occlusal surfaces greater as well as those of the periodontal ligament, which in turn results in higher level of bite force. They stated that average value of the MBF in healthy female subjects was 522.6 ± 25.01 N, and that in men it amounted to 811.8 ± 27.6 N. These findings are consistent with the results of our study. However, it is noteworthy that the MBF values obtained by different studies are difficult to compare. MBF value varies depending on the type of measuring instrument applied [27], the position of the measuring instrument within the dental arch, and the number of teeth included [28]. Therefore, the literature offers MBF values ranging from 388 N to 1,109 N [29, 30].

Based on the results of this study it was found that MBF was significantly higher in participants with bruxism compared to those without it, taking into account the gender difference. The findings of our study are consistent with the findings of the study conducted by Killiaridis et al. [31]. Some authors like Gibbs et al. [13] for instance, find that MBF in persons with bruxism was six times the one in those without it. However, Cosme et al. [15] did not find a significant difference between persons with bruxism and those without it, taking into account the gender difference between the subjects. Similar results were reported by some other authors, as well [32]. However, in these studies bite force was measured using a compressive transducer at the first molar region. Tortopidis et al. [33] addressed the issue of measuring instrument reliability and found that the variability in MBF values was highest when using a gnathodynamometer. The use of these measurement systems does not take into account OCA, which among other things can affect the results.

Our study shows no statistically significant difference in MBP/mm^2 between the persons with bruxism and those without it. Perhaps it is this segment of the research that indicates that masticatory muscles potential does not increase in patients with bruxism. However, the research shows that OCA is significantly higher in patients with bruxism, which is most probably due to teeth attrition and contact area expansions. According to Hatch et al. [34], bite force and the number of teeth in occlusion are determining factors in masticatory performance, whereas occlusal contacts determine 10–20% of MBF variation. Hidaka et al. [35] believe that

OCA, the number of occlusal contacts and the number of teeth present are significant determining factors for MBF. Increased levels of teeth clenching lead to greater intimacy between occlusal contacts in maxillary and mandibular dental arches. For example, with increasing teeth clenching/grinding levels from 30% to 100%, the occlusal contact areas are doubled. As our study shows, MBP/mm^2 was not significantly different between persons with bruxism and those without it, whereas OCA was significantly higher in persons with bruxism; therefore, the MBF was also higher.

Using a measuring system based on the prescale pressure sensitive sheet, Miyaura et al. [36] found that the bite potential closely correlated to the number of teeth present. Alkan et al. [37] monitored MBF values in persons with bruxism before and after stabilization splint treatment. They found that the occlusal contact area and bite force decline in patients using a splint for three months. Similar results were demonstrated by Kurita et al. [38] and Karakis et al. [39] In light of these data it is possible to comment on the muscle activity in relation to changes in the OCA and bite force.

The gold standard diagnostic method for bruxism is the use of polysomnographic recordings in a specialized sleep laboratory [40]. For the purposes of our study, the questionnaire and physical examination of the patients was used in the diagnosis of the patients with bruxism. Some studies compared clinical outcomes with the results of polysomnography to diagnose bruxism and found that the clinical criteria had a reliability of 83% in patients with bruxism and 81% in asymptomatic control subjects [41]. However, Baba et al. [42] did not find any associations between tooth wear status and ongoing bruxism. Therefore, an insufficient reliability of clinical methods in the diagnosis of bruxism may somewhat affect the results of this study.

Conclusion

Bruxism influences the increase of MBF. It also affects the increase in OCA, but not MBP. Therefore, registration of MBF can be used in the diagnosis and analysis of pathophysiological events during bruxism.

Gender is a significant determinant of bite force, which is why gender difference must be taken into account during analysis of MBF. These results may be considered as an initiative calling for further research for the sake of complete clarification of bruxism and its impact on the stomatognathic system.

R E F E R E N C E S

1. *Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ,* et al. Bruxism defined and graded: An international consensus. J Oral Rehabil 2013; 40(1): 2−4.
2. *Huynh N, Lavigne GJ, Okura K, Yao D, Adachi K.* Sleep bruxism. In: *Pasquale M, Sudhansu C*, editors. Sleep Disorder. Part II. Netherlands, Amsterdam: Elsevier; 2011.p. 901−11.
3. *Silverman S, Eversole LR, Trulove EL.* Essentials of Oral Medicine. Hamilton, London: BC Decker Inc; 2001.
4. *Bader G, Lavigne G.* Sleep bruxism; An overview of an oromandibular sleep movement disorder. Sleep Med Rev 2000; 4(1): 27−43.
5. *Manfredini D, Landi N, Tognini F, Montagnani G, Bosco M.* Occlusal features are not a reliable predictor of bruxism. Minerva Stomatol 2004; 53(5): 231−9.
6. *Manfredini D, Landi N, Romagnoli M, Bosco M.* Psychic and occlusal factors in bruxers. Aust Dent J 2004; 49(2): 84−9.
7. *Molina OF, dos Santos J Jr.* Hostility in TMD/bruxism patients and controls: A clinical comparison study and preliminary results. Cranio 2002; 20(4): 282−8.
8. *Lobbezoo F, Naeije M.* Etiology of bruxism: Morphological, pathophysiological and psychological factors. Ned TijdschrTandheelkd 2000; 107(7): 275−80. (Dutch)
9. *Castelo PM, Bonjardim LR, Pereira LJ, Gavião MB.* Facial dimensions, bite force and masticatory muscle thickness in preschool children with functional posterior cross-bite. Braz Oral Res 2008; 22(1): 48−54.
10. *Takeuchi N, Yamamoto T.* Correlation between periodontal status and biting force in patients with chronic periodontitis during the maintenance phase of therapy. J Clin Periodontol 2008; 35(3): 215−20.
11. *Lasilla V, Holmlund I, Koivumaa KK.* Bite force and its correlations in different denture types. Acta Odontol Scand 1985; 43(3): 127−32.
12. *Helkimo E, Ingervall B.* Bite force and functional state of the masticatory system in young men. Swed Dent J 1978; 2(5): 167−75.
13. *Gibbs CH, Mahan PE, Mauderli A, Lundeen HC, Walsh EK.* Limits of human bite strength. J Prosthet Dent 1986; 56(2): 226−9.
14. *Lyons MF, Baxendale RH.* A preliminary electromyographic study of bite force and jaw-closing muscle fatigue in human subjects with advanced tooth wear. J Oral Rehabil 1990; 17(4): 311−8.
15. *Cosme DC, Baldisserotto SM, Canabarrosde A, Shinkai RS.* Bruxism and voluntary maximal bite force in young dentate adults. Int J Prosthodont 2005; 18(4): 328−32.
16. *Nishigawa K, Bando E, Nakano M.* Quantitative study of bite force during sleep associated bruxism. J Oral Rehabil 2001; 28(5): 485−91.
17. *Nunes LM.* Association between bruxism and temporomandibular dysfunction. Bauru: School of Dentistry, University of Sao Paulo; 2003. (Brazilian)
18. *Molina OF, Junior S, Nelson SJ, Nowlin T.* A clinical study of specific signs and symptoms of CMD in bruxers classified by the degree of severity. Cranio 1999; 17(4): 268−79.
19. *Helkimo M.* Studies of function and dysfunction of the masticatory system. Index for anamnestic and clinical dysfunction and occlusal state. Sweden Dent 1974; 67(2): 101−21.
20. *Ferrario VF, Sforza C, Serrao G, Dellavia C, Tartagilia GM.* Single tooth bite forces in healthy young adults. J Oral Rehabil 2004; 31(1): 18−22.
21. *Pereira-Cenci T, Pereira LJ, Cenci MS, Bonachela WC, del Belcury AA.* Maximal bite force and its association with temporomandibular disorders. Braz Dent J 2007; 18(1): 65−8.
22. *Bonakdarchian M, Askari N, Askari M.* Effect of face form on maximal molar bite force with natural dentition. Arch Oral Biol 2009; 54(3): 201−4.
23. *Bakke M.* Bite force and occlusion. Semin Orthod 2006; 12(2): 120−6.
24. *Pizolato RA, Gavião MB, Berretin-Felix G, Sampaio AC, Trindade Junior AS.* Maximal bite force in young adults

temporomandibular disorders and bruxism. Braz Oral Res 2007; 21(3): 278−83.
25. *Raadsheer MC, van Eijden TM, van Ginkel FC, Prahl-Andersen B.* Contribution of jaw muscle size and craniofacial morphology to human bite force magnitude. J Dent Res 1999; 78(1): 31−42.
26. *Braun S, Freudenthaler JW, Hönigle K.* A study of maximum bite force during growth and development. Angle Orthod 1996; 66(4): 261−4.
27. *Olthoff LW, Van Der Glas HW, Van Der Blit A.* Influence of occlusal vertical dimension on the masticatory performance during chewing with maxillary splints. J Oral Rehabil 2007; 34(8): 560−5.
28. *Van Der Bilt A, Tekamp A, Van der Glas H, Abbink J.* Bite force and electromyograpy during maximum unilateral and bilateral clenching. Eur J Oral Sci 2008; 116(3): 217−22.
29. *Kogawa EM, Calderon PS, Lauris JR, Araujo CR, Conti PC.* Evaluation of maximal bite force in temporomandibular disorders patients. J Oral Rehabil 2006; 33(8): 559−65.
30. *Shinogaya T, Bakke M, Thomsen CE, Vilmann A, Matsumoto M.* Bite force and occlusal load in healthy young subjects: A methodological study. Eur J Prosthodont Restor Dent 2000; 8(1): 11−5.
31. *Killiaridis S, Johansson A, Haraldson T, Omar R, Carlsson GE.* Craniofacial morphology, occlusal traits, and bite force in persons with advanced occlusal tooth wear. Am J Orthod Dentofac Orthop 1995; 107(3): 286−91.
32. *Calderon Pdos S, Kogawa EM, Lauris JR, Conti PC.* The influence of gender and bruxism on the human maximum bite force. J Appl Oral Sci 2006; 14(6): 448−53.
33. *Tortopidis D, Lyons MF, Baxendale RH, Gilmour WH.* The variability of bite force measurement between sessions, in different positions within the dental arch. J Oral Rehabil 1998; 25(9): 681−6.
34. *Hatch JP, Shinkai RS, Sakai S, Rugh JD, Paunovich ED.* Determinants of masticatory performance in dentate adults. Arch Oral Biol 2001; 46(7): 641−8.
35. *Hidaka O, Iwasaki M, Saito M, Morimoto T.* Influence of clenching intensity on bite force balance, occlusal contact area, and average bite pressure. J Dent Res 1999; 78(7): 1336−44.
36. *Miyaura K, Morita M, Matsuka Y, Yamashita A, Watanabe T.* Rehabilitation of biting abilities in patients with different types of dental prostheses. J Oral Rehabil 2000; 27(12): 1073−6.
37. *Alkan A, Bulut E, Arici S, Sato S.* Evaluation of Treatments in Patients with Nocturnal Bruxism on Bite Force and Occlusal Contact Area. A preliminary report. Eur J Dent 2008; 2: 276−82.
38. *Kurita H, Ikeda K, Kurashina K.* Evaluation of the effect of a stabilization splint on occlusal force in patients with masticatory muscle disorders. J Oral Rehabil 2000; 27(1): 79−82.
39. *Karakis D, Dogan A, Bek B.* Evaluation of the effect of two different occlusal splints on maximum occlusal force in patients with sleep bruxism: A pilot study. J Adv Prosthodont 2014; 6(2): 103−8.
40. *Tosun T, Karabuda C, Cuhadaroglu* C. Evaluation of sleep bruxism by polysomnographic analysis in patients with dental implants. Int J Oral Maxillofac Implants 2003; 18(2): 286−92.
41. *Lavigne GJ, Rompre PH, Montplasir JY.* Sleep bruxism: Validity of clinical research diagnostic criteria in a controlled polysomnographic study. J Den Res 1996; 75(1): 546−52.
42. *Baba K, Haketa T, Clark GT, Ohyama T.* Does tooth wear status predict ongoing sleep bruxism in 30-year-old Japanese subject. Int J Prosthodont 2004; 17(1): 39−44.

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Impact of pharmacologic therapy for benign prostatic hyperplasia on prostate volume and free testosterone and consequently on urinary parameters and sexual desire in men

Uticaj farmakološke terapije benigne hiperplazije prostate na volumen prostate i slobodni testosteron i, posledično, na urinarne parametre i seksualnu želju muškaraca

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Abstract

Background/Aim. Pharmacologic therapy for benign prostatic hyperplasia (BPH) relieves disease progression and affects the androgen hormone status. A decrease in the level of free testosterone (freeT) within total testosterone (totalT) leads to symptoms of sexual dysfunction. The aim of this study was to show the impact of pharmacological treatment for BPH on prostate volume (PV) and levels of freeT and, consequently, on urinary parameters and sexual desire in men during 6 months of administration. **Methods**. This clinical prospective study included 156 BPH patients with moderate urinary symptoms – International Prostate Symptom Score (IPSS) < 19, PV > 30 mL and prostate specific antigen (PSA) value < 4 ng/mL. The average age of patients was 61.16 ± 2.97 years. The performed tests included determination of tumor markers (PSA, free PSA), hormones (totalT, freeT, freeT/totalT ratio), trans abdominal ultrasonography and uroflowmetry. Urinary symptoms were measured by IPSS and the Quality of Life (QoL) questionnaire while the changes in sexual desire were measured using the International Index of Erectile Function (IIEF) questionnaire. Four groups were formed, 39 patients each. The group 1 received alpha1-blocker (AB) tamsulosin, the group 2, 5 alpha-reductase inhibitor (5-ARI) finasteride, the group 3, combined therapy of both drugs (tamsulosin and finasteride), while the group 4 (control group) had no therapy. Follow-ups were performed every three and six months during therapy administration. **Results**. Prostate volume significantly decreased in the patients on combined therapy (-6.95 ± 2.00; $p < 0.001$) and finasteride (-6.67 ± 3.35). In the finasteride group, the levels of freeT (-4.23 ± 5.2; $p < 0.001$) and freeT/totalT ratio (-0.12 0.08; $p < 0.001$) significantly decreased as did the freeT (-2.64 ± 7.81) and freeT/totalT ratio (-0.09 ± 0.13) in the combined therapy group. Uroflowmetry showed a significant improvement in all parameters and all the therapy groups. Combined therapy provided the greatest improvement in the maximum flow rate (Qmax) (+4.06 ± 1.75; $p < 0.001$) and urinary symptoms (-10.95 ± 3.19). A significant improvement of sexual desire occurred in the patients on tamsulosin (+0.78 ± 1.00; $p < 0.001$), with a slight deterioration in the finasteride group, but without statistical significance. **Conclusion**. Hormonal component of pharmacologic therapy for BPH most effectively reduces PV and freeT levels, improves urinary symptoms with a slight decline of sexual desire in men on finasteride monotherapy.

Key words:
prostatic hyperplasia; drug therapy; urination disorders; sexual dysfunction, physiological; quality of life.

Apstrakt

Uvod/Cilj. Farmakološka terapija benigne hiperplazije prostate (BHP) ublažava progresiju bolesti i utiče na androgeni hormonski status. Opadanje nivoa slobodnog testosterona (slobodniT) unutar ukupnog testosterona (ukupniT) dovodi do simptoma seksualne disfunkcije. Cilj rada bio je da se prikažu rezultati uticaja farmakološke terapije BHP u toku 6 meseci korišćenja na volumen prostate (VP) i nivo slobodnogT kao i efekat na urianarne parametre i seksualnu želju muškaraca. **Metode**. Ova klinička prospektivna studija obuhvatila je 156 bolesnika sa BHP, sa umerenim simptomima poremećaja mokrenja – internacionalni prostata simptom skor (IPSS) < 19,VP > 30 mL i vrednošću prostata specifičnog antigena (PSA) < 4 ng/mL. Prosečna starost bolesnika bila je 61,16 ± 2,97 god. Rađene su analize krvi sa određivanjem tumorskih markera (PSA, slobodni PSA), hormona (ukupniT, slobodniT, odnos slobodniT/ukupniT), transabdominalna ultra-

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sonografija i urofloumetrija. Simptomi poremećenog mokrenja mereni su upitnicima za IPSS i kvalitet života (*Quality of Life* – QoL), a promene seksualne želje upitnikom Internacionalni indeks erektilne funkcije (IIEF). Formirane su četiri grupe sa po 39 bolesnika. Prva grupa dobijala je alfa1-blokator (AB) tamsulosin, druga grupa inhibitor 5alfa-reduktaze (5-ARI) finasterid, treća grupa kombinovanu terapiju (tamsulosin i finasterid) i četvrta, kontrolna grupa, bila je bez terapije. Kontrole su rađene na tri i šest meseci tokom terapije. **Rezultati**. Značajno je smanjen VP kod bolesnika sa kombinovanom terapijom (-6,95 ± 2,00; $p < 0,001$) i finasteridom (-6,67 3,35). U grupi sa finasteridom značajno je smanjen slobodniT (-4,23 ± 5,20; $p < 0,001$) i odnos slobodniT/ukupniT (-0,12 ± 0,08; $p < 0,001$), kao i u grupi sa kombinovanom terapijom [slobodniT (-2,64 ± 7,81) i odnos slobodniT/ukupniT (-0,09 ± 0,13)]. Urofloumetrija je pokazala značajno poboljšanje svih urinarnih parametara u svim terapijskim grupama. Kombinovanom terapijom postignuto je najveće poboljšanje maksimalnog protoka (Qmax)(+4,06 1,75; $p < 0,001$) i simptoma poremećenog mokrenja (-10,95 ± 3,19). Seksualna želja kod bolesnika sa tamsulosinom bla je značajno poboljšana (+0,78 1,00; $p < 0,001$), dok je u grupi sa finasteridom registrovano blago pogoršanje, ali bez statističke značajnosti. **Zaključak**. Hormonska komponenta farmakološke terapije za BHP najefikasnije smanjuje VP i nivo slobodnogT, poboljšava simptome poremećenog mokrenja sa blagim pogoršanjem seksualne želje muškaraca lečenih samo finasteridom.

Ključne reči:
prostata, hipertrofija; lečenje lekovima: mokrenje, poremećaji; seksualni poremećaji; kvalitet života.

Introduction

Benign prostatic hyperplasia (BPH) is the fourth most common disease in elderly men. It causes difficulties in urination and sexual function disorders. The occurrence and development of BPH are triggered by age and male sex hormones [1]. In men aged over 60 histological changes which indicate BPH are present in over 60%, while over 40% experience lower urinary tract symptoms (LUTS) [2]. Male sex hormones, testosterone, androstenedione and dehydroepiandrosterone (DHEA), are important for the formation of secondary sexual characteristics, the existence of libido, erection, and spermatogenesis process. Their influence on the development of BPH contributes to the occurrence and maintenance of LUTS [3].

The androgenic activity of testosterone is 10 times higher than that of androstenedione and 20 times higher than DHEA. Testosterone is present in plasma in free (freeT) and bound form. Free testosterone (2% of totalT), comes into contact with different cells in the body: muscle, brain, skin and hair cells, the prostate and other sex organs [4]. For the most part, bound testosterone is weakly bound to plasma albumin (38%) or strongly bound to beta globulin (60%) – sex hormone binding globulin (SHBG). Free testosterone plus albumin bound testosterone implies bioavailable testosterone, which easily enters cells and better reflects the bioactivity of testosterone. After the age of 20, there is a gradual decline in levels of totalT by 0.4% and by 1.2% in freeT *per* year [5]. If freeT starts to decrease in totalT, the symptoms of decreased sexual activity will occur. However, even in the oldest men, the blood level of testosterone is high enough to maintain libido and spermatogenesis [6].

Pharmacological treatment of BPH includes alpha 1-blockers (ABs) and 5 alpha-reductase inhibitors (5-ARIs), individually or together as a combination therapy [7]. ABs bind to alpha-receptors, relaxing the smooth muscles of the prostate and the bladder neck. They facilitate urination without affecting prostate size [8]. 5-ARIs block the conversion of inactive forms of testosterone into dehydrotestosterone (DHT), which has a 2–5 times higher affinity to androgen receptors than testosterone. Decreasing the level of DHT reduces the prostate volume (PV) thus improving urinary symptoms. At the same time, 5-ARI lead to changes in hormonal status which affects the elements of sexual function [9].

Many studies have examined the relationship between male sex hormones and BPH, but only a few analyzed the relationship between free T and LUTS in BPH finding no significant interrelationships [10].

The aim of this paper was to demonstrate the impact of pharmacologic therapy for BPH on PV and the levels of freeT during 6 months of administration and the effects of such changes on LUTS, functional urinary parameters and sexual desire in men.

Methods

A prospective clinical study was conducted at the Military Hospital in Niš with the participation of 156 BPH patients. The average age of the entire cohort of patients was 61.16 ± 2.97 years, all were in good general condition, previously not treated for BPH. The patients were informed about the course and research goals and they all signed the consent form to participate in the research. The Ethics Committee of the Military Hospital in Niš approved the implementation of the study.

The research included patients without indications for surgical treatment, with moderate urinary symptoms [7 < International Prostate Symptom Score (IPSS) < 19], PV > 30 mL and the prostatic specific antigen (PSA) value of < 4 ng/mL. Patients with residual urine > 200 mL, infections, calculosis, malfunction of the kidneys and those who had undergone prostate biopsy for suspected malignacy were excluded.

After a general check-up and a digital rectal examination of the prostate, determination of serum tumor markers (PSA, freePSA), sexual hormones – testosteron (totalT, freeT, freeT/totalT ratio), creatinine, urine and urine culture with antibiogram were performed in all the patients. Prostate volume and post-void residual (PVR) urine were measured by transabdominal ultrasonography. The functionality of the lower urinary tract was measured using uroflowmetry parameters: Qmax, average flow rate (Qave), voiding time (VT), PVR with voided volume (Vcomp) of > 150 mL.

Urinary symptoms were measured using the IPSS and Quality of life (QoL) questionnaire. The IPSS is an 8-item questionnaire, consisting of seven symptom questions in the past month and QoL question. Four questions refer to voiding symptoms (incomplete emptying, intermittency, straining, weak stream) while three deal with storage symptoms (frequency, urgency, nocturia). The effect of hormonal changes was measured by the changes in the level of sexual desire and orgasmic function as provided by the International Index of Erectile Function (IIEF) questionnaire which assesses sexual function in the previous month. The questions include: how often did you feel sexual desire and how do you rate your level of sexual desire? The following questions were used to measure the orgasmic function: how often did you ejaculate during sexual acitivity and how often did you experience an orgasm? To assess the ejaculatory function three items were used for rating the domains of ejaculation (frequency, strength and volume) as given in the Male Sexual Health Questionnaire-Ejaculatory Dysfunction (MSHQ-EjD) [11].

The patients completed the questionnaires before treatment. After three and six months of the therapy, follow-up examinations were performed like the ones performed upon entering the study. Based on the results, four groups of 39 patients were formed. The first group received AB tamsulosin 0.4mg/day, the second group received 5-ARI finasteride 5 mg daily while the third group received combination therapy (tamsulosin andfinasteride). The fourth control group consisted of patients with mild symptoms (IPSS < 7), without therapy, but provided advice on lifestyle (self-management as a part of watchful waiting). Tamsulosin was given to patients with PV < 40 mL, finasteride for PV 40–50 mL and combination therapy to patients with PV > 50 mL. Average values of urinary symptoms intensity did not differ significantly across the two groups.

Two patients from the group on tamsolusin and another two on combination therapy did not undergo the third examination. The reasons for this were of objective nature – two patients had changed their residence, the third was recovering after a road accident, while the fourth was receiving inpatient orthopedic treatment. This was taken into consideration in statistical processing.

Testing the differences between the questionnaire scores and variables before the examination, three and six months after the therapy was performed using repeated measure analysis of variance (RM ANOVA). The respondents' ages within the groups were compared using unilateral analysis of variance (one-way ANOVA) and the Tukey *post-hoc* test.

The questionnaire scores and variables were presented as mean values ± standard deviation ($\bar{x} \pm SD$). The level of statistical significance was $p < 0.05$.

Results

The average age was no statistically significantly different between the formed groups. In the group using tamsulosin it was 60.69 ± 3.22 years, 61.56 ± 3.30 years in the finasteride group, 61.76 ±2.51 years in the combination therapy group and 60.64 ± 2.70 years in the control group.

Table 1 shows changes in the parameters measured

Table 1

The values of variables during the study in the therapy groups

Therapy group	Variables	Testing (mean ± SD)			Comparison between testing *(p)*		
		Baseline (I)	After 3 mths (II)	After 6 mths (III)	I *vs* II	II *vs* III	I *vs* III
Tamsulosin	PSA (ng/mL)	1.08 ± 0.45	0.97 ± 0.48	0.87 ± 0.48	0.031*	0.097	< 0.001*
	Free PSA (ng/mL)	0.32 ± 0.19	0.28 ± 0.18	0.31 ± 0.15	0.134	0.109	0.450
	TotalT (ng/mL)	5.21 ± 1.83	5.44 ± 2.02	5.08 ± 1.96	0.531	0.169	0.704
	FreeT (pg/mL)	14.94 ± 7.18	13.37 ± 4.09	13.4 ± 6.19	0.137	0.874	0.215
	FreeT/TotalT (%)	0.30 ± 0.14	0.28 ± 0.13	0.29 ± 0.14	0.498	0.549	0.843
	Voiding time (s)	49.61 ± 21.89	45.00 ± 18.82	41.19 ± 14.58	0.128	0.133	0.010*
	Qmax (mL/s)	13.66 ± 3.19	15.54 ± 3.26	16.49 ± 3.19	< 0.001*	0.007*	< 0.001*
	Qave (mL/s)	8.69 ± 2.41	9.36 ± 2.44	9.84 ± 2.66	0.022*	0.063	0.003*
	PVR (mL)	40.44 ± 29.00	30.10 ± 21.29	23.30 ± 17.83	0.006*	0.043*	< 0.001*
Finasteride	PSA (ng/mL)	1.94 ± 0.79	1.25 ± 0.56	1.01 ± 0.48	< 0.001*	< 0.001*	< 0.001*
	Free PSA (ng/mL)	0.45 ± 0.22	0.25 ± 0.20	0.23 ± 0.14	< 0.001*	0.255	< 0.001*
	TotalT (ng/mL)	5.05 ± 1.97	6.35 ± 2.48	6.40 ± 3.41	< 0.001*	0.896	0.002*
	FreeT (pg/mL)	12.89 ± 4.27	11.94 ± 6.17	8.66 ± 5.57	0.243	0.001*	< 0.001*
	FreeT/TotalT (%)	0.26 ± 0.08	0.19 ± 0.09	0.14 ± 0.09	< 0.001*	< 0.001*	< 0.001*
	Voiding time (s)	53.03 ± 19.70	43.55 ± 17.73	41.26 ± 14.77	0.002*	0.328	< 0.001*
	Qmax (mL/s)	12.85 ± 2.59	14.54 ± 2.43	16.52 ± 2.71	< 0.001*	< 0.001*	< 0.001*
	Qave (mL/s)	7.93 ± 2.39	9.13 ± 2.84	10.14 ± 2.82	< 0.001*	0.004*	< 0.001*
	PVR (mL)	52.03 ± 26.32	33.54 ±19.24	18.54 ± 12.09	< 0.001*	< 0.001*	< 0.001*
Combination therapy	PSA (ng/mL)	2.31 ± 0.95	1.53 ± 0.91	1.22 ± 0.71	< 0.001*	< 0.001*	< 0.001*
	Free PSA (ng/mL)	0.54 ± 0.24	0.30 ± 0.20	0.28 ± 0.16	< 0.001*	0.333	< 0.001*
	TotalT (ng/mL)	5.61 ± 1.85	6.33 ± 2.65	6.46 ± 2.67	0.018*	0.784	0.051
	FreeT (pg/mL)	13.83 ± 5.91	12.43 ± 6.62	11.17 ± 7.18	0.203	0.080	0.047*
	FreeT/TotalT (%)	0.26 ± 0.11	0.20 ± 0.08	0.17 ± 0.10	0.002*	0.013*	< 0.001*
	Voiding time (s)	58.09 ± 22.34	51.75 ± 17.02	40.93 ± 12.43	0.033*	< 0.001*	< 0.001*
	Qmax (mL/s)	12.18 ± 2.60	14.44 ± 3.00	16.05 ± 2.92	< 0.001*	< 0.001*	< 0.001*
	Qave (mL/s)	7.11 ± 2.23	8.41 ± 2.46	9.42 ± 2.80	< 0.001*	< 0.001*	< 0.001*
	PVR (mL)	64.51 ± 26.23	34.77 ± 21.73	21.03 ± 14.52	< 0.001*	< 0.001*	< 0.001*

*** – statistically significant difference; SD – standard deviation; PSA – prostate specific antigen; TotalT – total testosterone; FreeT – free testosterone; PVR – post-void residual urine; Qmax – maximum flow rate; Qave – average flow rate; mths – months.**

during research. Assessment of prostate size (mL) by ultrasound showed a significant reduction in PV after 6 months of the treatment in the group on combination therapy (-6.95 ± 2.00) and the finasteride users (-6.67 ± 3.35). In the patients using only tamsulosin there were no significant changes (Figure 1).

PSA (ng/mL) significantly decreased in all therapy groups after 6 months, the most in patients on combination therapy (-1.15 ± 0.51) followed by the groups on finasteride (-0.93 ± 0.64) and tamsulosin (-0.21 ± 0.31). Free PSA (ng/mL) significantly decreased with combination therapy (-0.27 ± 0.15) and finasteride (-0.22 ± 0.14), while non-significant decrease occurred in the tamsulosin group.

Regarding the impact on hormones the significant change of totalT levels (ng/mL) from the baseline values occurred only in the finasteride group, where it was increased (+1.34 ± 2.49). Free testosterone (pg/mL) was significantly reduced in the groups on finasteride (-4.23 ± 5.20), and combination therapy of (-2.64 ± 7.81), while non-significant changes were recorded in the tamsulosin group. Free testosterone/total testosterone ratio (%) as a measure of androgen bioactivity of total testosterone decreased in all groups after 6 months of therapy, with statistical significance only in groups on finasteride (-0.12 ± 0.08) and combination therapy (-0.09 ± 0.13) (Table 1).

Uroflowmetry results showed a significant improvement of all parameters in all therapy groups compared to baseline. Voiding time (sec) decreased the most with combination therapy (-18.51 ± 17.77), then with finasteride (-11.77 ± 16.58) and the tamsulosin (-8.61 ± 19.14). The highest increase of the Qmax (mL/s) was registered in the combination therapy group (+4.06 ± 1.75), followed by the finasteride (+3.67 ± 1.84) and the tamsulosin group (+2.81 ± 2.61) (Table 2). The highest increase of the Qave (mL/s) was in the combination therapy group (+2.47 ± 1.84), followed by the groups on finasteride (+2.21 ± 2.12) and tamsulosin (+1.16 ± 2.21). Post-void residual urine (mL) decreased mostly with combination therapy (-44.22 ± 26.02), then with finasteride (-33.49 ± 26.06) and the least with tamsulosin (-16.08 ± 25.22).

Urinary symptoms (IPSS) significantly improved in all therapy groups as follows: (-10.95 ± 3.19) with combination therapy, (-9.00 ± 2.84) with finasteride and (-5.84 ± 3.08) with tamsulosin (Table 2). Quality of life also improved significantly in all the three therapy groups, the most with combined therapy (-2.95 ± 0.97), then with finasteride (-2.85 ± 1.01) and tamsulosin (-2.32 ± 1.00) (Table 2).

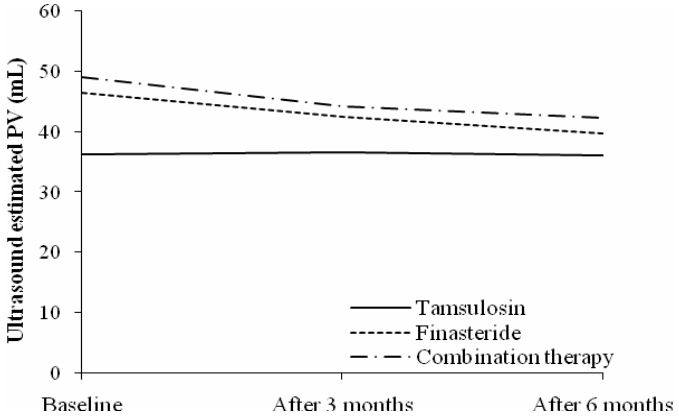

Fig. 1 – The prostate volume (PV) on ultrasound scan during the study.

Table 2

Score values of urinary symptoms and QoL during the study in all the groups

Therapy group	Score	Testing (Mean ± SD)			Comparison between testing *(p)*		
		Baseline (I)	After 3 mths (II)	After 6 mths (III)	I vs II	II vs III	I vs III
Tamsulosin	IPSS	13.64 ± 3.35	9.21 ± 2.66	7.51 ± 2.66	< 0.001*	0.001*	< 0.001*
	Voiding symptoms	7.79 ± 2.65	4.74 ± 2.06	3.70 ± 2.22	< 0.001*	0.010*	< 0.001*
	Storage symptoms	6.10 ± 1.67	4.51 ± 1.17	3.81 ± 1.29	< 0.001*	0.001*	< 0.001*
	QoL	3.74 ± 0.75	1.79 ± 0.89	1.32 ± 0.85	< 0.001*	0.006*	< 0.001*
Finasteride	IPSS	16.69 ± 2.91	10.97 ± 2.45	7.69 ± 2.62	< 0.001*	< 0.001*	< 0.001*
	Voiding symptoms	9.21 ± 2.33	6.03 ± 1.94	3.69 ± 1.78	< 0.001*	< 0.001*	< 0.001*
	Storage symptoms	7.49 ± 1.71	4.95 ± 1.45	3.97 ± 1.46	< 0.001*	< 0.001*	< 0.001*
	QoL	4.10 ± 0.64	1.97 ± 0.84	1.26 ± 0.79	< 0.001*	< 0.001*	< 0.001*
Combination therapy	IPSS	19.82 ± 3.09	12.05 ± 2.65	8.89 ± 2.60	< 0.001*	< 0.001*	< 0.001*
	Voiding symptoms	10.82 ± 2.48	6.41 ± 2.06	4.03 ± 1.91	< 0.001*	< 0.001*	< 0.001*
	Storage symptoms	9.26 ± 2.57	5.62 ± 1.63	4.84 ± 1.68	< 0.001*	< 0.001*	< 0.001*
	QoL	4.33 ± 0.70	2.10 ± 1.02	1.41 ± 0.86	< 0.001*	< 0.001*	< 0.001*
Control group	IPSS	7.21 ± 1.28		7.05 ± 1.39			0.509
	Voiding symptoms	3.49 ± 1.50		3.36 ± 1.44			0.463
	Storage symptoms	3.72 ± 1.17		3.69 ± 1.08			0.875
	QoL	1.95 ± 0.94		1.56 ± 0.79			0.017*

*** – statistically significant difference; SD – standard deviation; IPSS – International Prostate Symptom Score; QoL – Quality of Life; mths – months.**

Voiding symptoms referred to in IPSS significantly improved in all the therapy groups. The greatest improvement occured with combination therapy (-6.86 ± 2.85), followed by finasteride (-5.51 ± 2.30) and tamsulosin treatment (-3.95 ± 2.17). Storage symptoms referred to in IPSS also improved significantly in all the therapy groups. The greatest improvement was reported in the combined therapy group (-4.38 ± 2.47), followed by the finasteride group (-3.51 ± 1.50) and, finally, the tamsolusin group (-2.16 ± 1.57).

Measurement of the effects of hormonal changes on sexual desire demonstrated the presence of significant improvement in patients submitted to tamsulosin at the end of the treatment (+0.78 ± 1.00), recording no significant changes in the other two therapy groups.

Pharmacologic therapy of BPH produced significant side effects on sexual desire in patients by disrupting their orgasmic function and EjD (Table 3). A significant disruption of orgasmic function was most prominent in the group on tamsulosin (-1,03 ± 1,94), followed by the group on combined therapy (-0.76 ± 2.07), while in the finasteride group this change was insignificant (-0.54 ± 1.68).

Significant EjD was reported in all the therapy groups. It was most prominent in the group receiving tamsulosin (-4.38 ± 2.55), with the decline in the ejaculatory function in 25 (64%) patients, whereas no change was recorded in 14 (36%) patients. This is followed by the combined therapy group (-3.89 ± 2.84) and the decline of function in 23 (59%) patients, no changes in 13 (33%) patients and the improvement in 3 (8%) patients. In the finasteride group (-1.49 ± 2.52) the decline was recorded in 15 (38%) patients, no changes were found in 19 (49%) patients while the improvement was reported by 5 (13%) patients.

The complete absence of ejaculation was recorded in the group receiving combined therapy in 9 patients (23%), in 6 patients (15%) being administered tamsulosin and in 2 patients (5%) on finasteride.

The control group showed the improvement in Qmax ($p = 0.002$), QoL ($p = 0.017$) and sexual desire ($p = 0.005$), although this was not a standard control group as the patients were given recommendations on lifestyle adjustments. The values of other scores did not change significantly (Table 4).

Six months of administration of pharmacologic therapy for BPH treatment is considered short time. There were no patients with the acute urine retention during therapy, nor were they in need of prostate surgery.

Discussion

A decrease in PV by the end of the study in the groups on finasteride (by 14.4%) and combination therapy (14.2%) indicates their almost identical therapeutic effect. In patients using only tamsulosin no significant changes occurred as the drugs from the AB group do not affect the growth of prostatic glandular tissue. Alleviating subvesical obstruction by reducing PV lead to a significant increase of the Qmax and Qave. The effect of finasteride also resulted in a greater reduction in VT and PVR compared to the same effect reported for the tamsulosin group.

Table 3

Score values of sexual desire, orgasmic function and ejaculatory dysfunction (EjD) during the study across therapy groups

Therapy group	Score	Testing (mean ± SD)			Comparison between testing *(p)*		
		Baseline (I)	After 3 mths (II)	After 6 mths (III)	I *vs* II	II *vs* III	I *vs* III
Tamsulosin	Sexual desire	6.69 ± 1.49	7.31 ± 1.47	7.46 ± 1.41	0.005*	0.554	< 0.001*
	Orgasmic function	8.36 ± 1.88	7.67 ± 1.84	7.35 ± 1.72	0.043*	0.066	0.003*
	EjD	10.49 ± 2.43	7.46 ± 2.67	6.22 ± 2.31	< 0.001*	0.001*	< 0.001*
Finasteride	Sexual desire	6.56 ± 1.52	6.41 ± 1.50	6.03 ± 1.46	0.504	0.141	0.053
	Orgasmic function	7.92 ± 2.02	8.00 ± 1.45	7.38 ± 2.07	0.760	0.010	0.053
	EjD	9.26 ± 2.68	8.49 ± 2.13	7.77 ± 2.50	0.004*	0.035*	0.001*
Combination therapy	Sexual desire	5.92 ± 1.68	6.10 ± 1.55	6.19 ± 1.60	0.382	0.918	0.368
	Orgasmic function	6.79 ± 2.25	6.10 ± 2.09	5.97 ± 2.32	0.048*	0.650	0.033*
	EjD	8.56 ± 3.08	5.77 ± 2.75	4.59 ± 2.50	< 0.001*	< 0.001*	< 0.001*

*** *p* – statistically significant difference; SD – standard deviation; mths – months.**

Table 4

The values of variables during the study in the control group

Variables	Testing (mean ± SD)		Comparison between testing *(p)*
	Baseline	After 6 mths	
PSA (ng/mL)	1.09 ± 0.48	0.97 ± 0.75	0.240
Free PSA (ng/mL)	0.29 ± 0.15	0.27 ± 0.15	0.302
TotalT (ng/mL)	4.40 ± 1.60	4.59 ± 1.82	0.505
FreeT (pg/mL)	12.31 ± 4.57	13.19 ± 5.40	0.181
FreeT/TotalT (%)	0.66 ± 2.28	1.00 ± 3.09	0.302
Voiding Time (s)	39.80 ± 12.40	41.13 ± 12.08	0.468
Qmax (mL/s)	18.40 ± 4.01	19.65 ± 4.68	0.002*
Qave (mL/s)	10.62 ± 2.98	10.62 ± 2.41	0.989
PVR (mL)	20.28 ± 17.44	19.59 ± 15.97	0.780
Sexual desire	6.92 ± 1.94	7.56 ± 1.60	0.005*

*** *p* – statistically significant difference; SD – standard deviation.**
For abbreviations see under Table 1.

The largest decline in PSA and fPSA levels in the groups on combination therapy and finasteride was expected because they accompany the enlarged volume of hyperplastic tissue. The decreased PSA levels in the tamsulosin group may be explained by relieved voiding and less irritation of the prostate tissue caused by reducedi ntravesical pressure and pressure in the prostatic urethra during urination.

The Proscar Long-Term Efficacy and Safety Study (PLESS) investigated the effects of finasteride during 4 years. Finasteride reduced PV by 17.9% compared to its increase of 14.1% in the placebo group. Urinary flow significantly improved with a decreased risk of surgical treatment by 55% [12]. New analysis of Medical Therapy of Prostatic Symptoms (MTOPS) studies shows the significant reduction of clinical progression of BPH by finasteride with PV > 30 mL (decrease of 5.79 mL), and with no significant effects in patients with PV < 30 mL (an increase of 0.28 mL) [13].

In a study by Zabkowski [14] finasteride led to a decrease in PV up to 40% after 12 months. In their 5-year retrospective analysis of 5-ARI effects, Kaplan et al. [15] pointed out that finasteride and other 5-ARI dutasteride, are similarly effective in reducing PV and improving Qmax and LUTS in BPH patients after 12 months of administration. Dutasteride significantly reduces PSA and PV after just 3 months of the therapy.

Preclinical studies show that the new GhRh and LhRh antagonists can cause a decrease in PV by direct inhibitory action of GhRh and LhRh (growth hormone-releasing hormone and luteinizing hormone-relating hormone, respectively) antagonists *via* prostate receptors [10]. It is believed that the IPSS is significantly correlated with age, PV and totalT, but not with freeT or serum levels of other sex hormones [16]. The mean PSA level and the average PV significantly increase with age. The mean level of PSA increases about 0.3 ng/mL every 10 years [17].

Dihydrotestosterone levels increase after the age of 40 due to the inability of peripheral tissue to use freeT which remains in serum in DHT form. The values of freeTand freeT/totalT ratio were significantly reduced in the groups on finasteride and combination therapy. However, this did not significantly change the level of sexual desire after six months of the therapy. In two groups of patients who had used finasteride as monotherapy or as a part of combination therapy, a total of 41% had slight deterioration in sexual desire. Extremely decreased sexual desire (from the previous normal or mild dysfunction to the level of difficult function or the occasional occurrence of sexual desire) was reported by 7.7% of the patients, all in the finasteride group. Combination therapy slightly improved sexual desire probably due to the positive effect of tamsulosin on erectile function. There was no single case of complete absence of sexual desire. Only the group using tamsulosin experienced a significant improvement in sexual desire with significant improvement of urination. Opinions are divided over whether AB therapy leads to the improvement of sexual desire by improving LUTS and consequently the erectile function or whether the treatment affects the two processes separately [18].

Gur et al. [19] state that by reducing the DHT levels, finasteride results in a decrease or loss of sexual desire in 2–10%. Other data show that after 12 months of finasteride use, DHT levels decreased by 80% with no significant changes in serum testosterone while increasing Qmax by 1.6%, and decreasing the IPSS by 2.7% [9].

Primary side effects of pharmacologic treatment for BPH are decreased ejaculation and aggravated orgasmic function. They are most prevalent in the tamsulosin group and manifest themselves during sexual activity as the reduced number of ejaculations and the decreased ejaculatate volume or the complete absence thereof. The findings of other studies show that the administration of AB over a longer period leads to the higher incidence of EjD, while the combined therapy possess a three-times higher risk of EjD compared to that of an AB or 5-ARI monotherapy [20].

In the finasteride taking group, the aggravation of ejaculation occured mostly due to decreased ejaculate force. Sexual desire did not decrease significantly, as reported in major studies where administration of 5-ARI resulted in the loss of sexual desire in 2–10% and EjD in 0–8% [21]. Trost et al. [22] note that sexual desire decreases in 1.5% and EjD in 3.4% with the use of 5-ARI compared to that of the placebo.

Beneficial effect of 5-ARI on prostate is decreasing the risk of lower grade prostate cancer by changing the metabolism of androgens [9]. Despite the belief that androgens are necessary for the development and growth of the prostate, new epidemiological studies state that changes in androgen serum concentrations do not affect the processes within the gland regulated by androgens and that in older men the influence of androgens on PV and LUTS are not in harmony [3]. Only the age correlates with BPH (with a prevalence of 8% in the fourth decade to >70% in the seventh decade of life), while the potential effect of testosterone on LUTS may be indirect [23]. Kim et al. [24] reported that totalT level significantly decreased in patients with ≥ 4 episodes of nocturia and are significantly associated with the presence of expressed LUTS.

The efficacy of combination therapy compared to AB and 5-ARI monotherapy demonstrates significantly greater improvement in functional parameters and voiding symptoms by unifying common characteristics of action of two classes of drugs. The results of the recent studies demonstrated that the combination therapy leads to significant reduction of PV, PSA and IPSS and improvement of Qmax. The best effect in reducing the progression of BPH is seen in PV > 35mL and PSA > 2.0 ng/mL [25]. Symptoms improved by combination therapy and maintained after discontinuation of AB and continuous administration of 5-ARI monotherapy, whereas the risk of urinary retention due to the re-growth of the prostate is reduced [26].

Hormonal component of the pharmacological therapy in the form of finasteride as a monotherapy or as a part of combination therapy significantly reduced PV. It improved functional urinary parameters and alleviated difficulties with urination. At the same time the level of freeT and freeT/totalT ratio was reduced which led to a slight deterioration of sexual desire only in the patients from the finasteride group.

The limitations of this study lie in the fact that it is centred around a small number of respondents and that it involves a brief treatment period of six months with the absence of remote therapeutic results.

Standard pharmacologic treatment of BPH is still based on AB, 5-ARI and their combination [27]. Having in mind the preliminary findings of this paper and the findings of most other studies, this type of treatment should be personalized in the future according to the type of symptoms, the presence of sexual dysfunction and the risk of BPH progression. Patients need to be informed about any side effects the drugs might have on their sexual function, particularly if they are younger men and thus include them in the decision-making process regarding their treatment.

Conclusion

The use of pharmacologic therapy for BPH reduces the intensity of urinary symptoms and improves the QoL. The finasteride based therapy, as monotherapy or combination therapy, by the reduction of the PV may probably efficiently alleviate the disease progression. Androgenic hormone status is affected by the reduction of freeT levels which if used as finasteride monotherapy may slightly deteriorate sexual desire in men.

R E F E R E N C E S

1. *Patel ND, Parsons JK.* Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. Indian J Urol 2014; 30(2): 170−6.
2. *Kirby R.* Improving lower urinary tract symptoms in BPH. Practitioner 2011; 255(1739): 15−9, 2.
3. *Cooper LA, Page ST.* Androgens and prostate disease. Asian J Androl 2014; 16(2): 248−55.
4. *Buvat J, Maggi M, Guay A, Torres LO.* Testosterone deficiency in men: systematic Review and standard operating procedures for diagnosis and treatment. J Sex Med 2013; 10: 245−84.
5. *Maggi M, Buvat J, Corona G, Guay A, Torres LO.* Hormonal causes of male sexual dysfunctions and their management (hyperprolactinemia, thyroid disorders, GH disorders, and DHEA). J Sex Med 2013; 10: 661−77.
6. *Seisen T, Rouprêt M, Gallais JL, Costa P.* Relevant clinical and biological criteria for the diagnosis of androgen deficiency in the aging male (ADAM). Prog Urol 2012; 22(Suppl 1): S21−6. (French)
7. *Russo A1, La Croce G, Capogrosso P, Ventimiglia E, Colicchia M, Serino A*, et al. Latest pharmacotherapy options for benign prostatic hyperplasia. Expert Opin Pharmacother 2014; 15(16): 2319−28.
8. *Osman NI, Mangera A, Chapple CR.* Non-Hormonal treatment of BPH/BOO. Indian J Urol 2014; 30(2): 194−201.
9. *Azzouni F, Mohler J.* Role of 5α-reductase inhibitors in benign prostatic diseases. Prostate Cancer Prostatic Dis 2012; 15(3): 222−30.
10. *Rick FG, Saadat SH, Szalontay L, Block NL, Kazzazi A, Djavan B,* et al. Hormonal manipulation of benign prostatic hyperplasia. Curr Opin Urol 2013; 23(1): 17−24.
11. *Giuliano F.* Questionnaires in sexual medicine. Prog Urol 2013; 23(9): 811−21.
12. *Paick S, Meehan A, Lee M, Penson D, Wessells H.* The relationship among lower urinary tract symptoms, prostate specific antigen and erectile dysfunction in men with benign prostatic hyperplasia: results from the proscar long-term efficacy and safety study. J Urol 2005; 173(3): 903−7.
13. *Kaplan SA, Lee JY, Meehan AG, Kusek JW*, MTOPS Research Group. Long-term treatment with finasteride improves clinical progression of benign prostatic hyperplasia in men with an enlarged versus a smaller prostate: Data from the MTOPS trial. J Urol 2011; 185(4): 1369−73.
14. *Zabkowski T.* Evaluation of the clinical indications, adverse drug reactions, and finasteride use in patients with benign prostatic hyperplasia in Poland. Pharmacol Rep 2014; 66(4): 565−9.
15. *Kaplan SA, Chung DE, Lee RK, Scofield S, Te AE.* A 5-year retrospective analysis of 5alpha-reductase inhibitors in men with benign prostatic hyperplasia: Finasteride has comparable urinary symptom efficacy and prostate volume reduction, but less sexual side effects and breast complications than dut. Int J Clin Pract 2012; 66(11): 1052−55.
16. *Favilla V, Cimino S, Castelli T, Madonia M, Barbagallo I, Morgia* G. Relationship between lower urinary tract symptoms and serum levels of sex hormones in men with symptomatic benign prostatic hyperplasia. BJU Int 2010; 106(11): 1700−3.
17. *Park DS, Hong JY, Hong YK, Lee SR, Hwang JH, Kang MH,* et al. Correlation between serum prostate specific antigen level and prostate volume in a community-based cohort: large-scale screening of 35, 223 Korean men. Urology 2013; 82(6): 1394−9.
18. *Welliver C, Butcher M, Potini Y, McVary KT.* Impact of alpha blockers, 5-alpha reductase inhibitors and combination therapy on sexual function. Curr Urol Rep 2014; 15(10): 441.
19. *Gur S, Kadowitz PJ, Hellstrom WJ.* Effects of 5-alpha reductase inhibitors on erectile function, sexual desire and ejaculation. Expert Opin Drug Saf 2013; 12(1): 81−90.
20. *Gacci M, Eardley I, Giuliano F, Hatzichristou D, Kaplan SA, Maggi M*, et al. Critical Analysis of the Relationship Between Sexual Dysfunctions and Lower Urinary Tract Symptoms Due to Benign Prostatic Hyperplasia. Eur Urol 2011; 60(4): 809−25.
21. *Irwig MS.* Persistent sexual side effects of finasteride: Could they be permanent. J Sex Med 2012; 9(11): 2927−32.
22. *Trost L, Saitz TR, Hellstrom WJ.* Side effects of 5-alpha reductase inhibitors A comprehensive review. Sex Med Rev 2013; 1: 24−41.
23. *Lee JH, Kim Y, Park YW, Lee DG.* Relationship between benign prostatic hyperplasia/lower urinary tract symptoms and total serum testosterone level in healthy middle-aged eugonadal men. J Sex Med 2014; 11(5): 1309−15.
24. *Kim MK, Zhao C, Kim SD, Kim DG, Park JK.* Relationship of sex hormones and nocturia in lower urinary tract symptoms induced by benign prostatic hyperplasia. Aging Male 2012; 15(2): 90−5.
25. *Joo KJ, Sung WS, Park SH, Yang WJ, Kim TH.* Comparison of α-blocker monotherapy and α-blocker plus 5α-reductase inhibitor combination therapy based on prostate volume for treatment of benign prostatic hyperplasia. J Int Med Res 2012; 40(3): 899−908.
26. *Son H, Cho SY, Park S, Kang JY, Kim CS, Kim HG.* A retrospective study of clinical outcomes of α-blocker or finasteride monotherapy followed by combination therapy: determination of the period of combination therapy of α-blocker and finasteride. Int J Clin Pract 2013; 67(4): 351−5.
27. *Bechis SK, Otsetov AG, Ge R, Olumi AF.* Personalized Medicine for the Management of Benign Prostatic Hyperplasia. J Urol 2014; 192(1): 16−23.

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SHORT COMMUNICATIONS

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Breast augmentation with silicone implants performed without drainage – retrospective analysis of 726 cases

Uvećanje dojki silikonskim implantima bez drenaže – retrospektivna analiza 726 pacijentkinja

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Abstract

Background/Aim. Breast augmentation has been one of the most popular aesthetic procedures. Early complications, like infection, seroma, hematoma and capsular contracture like ones of the most frequent long term complications, might be related to wound drainage. The aim of the study was to investigate the rate of the complications of breast augmentation procedure performed without drainage. **Methods.** Retrospective analysis of all patients who had underwent breast augmentation in the period of 2003–2013 was performed. From the medical history of the patients, data related to their demographic characteristics, surgical technique and the rate of complications were collected. Wound drainage had not been used in any of the patients. The patients were followed at the discharge, after 7 days, three months and yearly thereafter. Wound seroma, wound hemathoma, wound infection and capsular contracture were followed. **Results.** There were 726 patients with the average age of 28.5 (22–48) years. Breast augmentation using silicone implants was performed with inframammary approach using subglandular and submuscular technique. The average implant size was 339 (200–520) cc. Subglandular augmentation had 545 (75%) of the patients while 181 (25%) received an implant in submuscular plane: completely submuscularly in 95/726 (13%) and by dual plane technique in 86/726 (12%) of the patients. In early postoperative period, there were no infection, five (0.7%) seromas and eight (1.1%) hematoma (five of them required surgical evacuation). There was no statistically significant difference between the two surgical techniques in terms of complication rate. During follow-up, there were three (0.4%) capsular contractures. **Conclusion.** The incidence of complications in our group of patients after breast augmentation is low even though no drainage was used. Still, further randomized trials are needed to prove the role of drainage in prevention of complications after breast augmentation.

Key words:
mammoplasty; breast implants; drainage; postoperative complications.

Apstrakt

Uvod/Cilj. Uvećanje dojki jedna je od najčešće izvođenih operacija u estetskoj hirurgiji. Rane komplikacije kao što su infekcija, serom, hematom i kontraktura kapsule, kao i neke kasne komplikacije mogu biti posledice drenaže rane. Cilj ove studije bio je da ispita učestalost komplikacija nakon operacije uvećanja dojki izvedene bez drenaže rane. **Metode.** Učinjena je retrospektivna analiza svih bolesnica podvrgnutih uvećanju dojki u periodu 2003–2013. Iz medicinske dokumentacije bolesnica dobijeni su podaci o demografskim karakteristikama, hirurškoj tehnici i broju komplikacija. Drenaža rane nije rađena u ovoj grupi bolesnica koje su praćene na otpustu, nakon 7 dana, tri meseca i godinu dana posle operacije. Praćena je učestalost seroma rane, hematoma, infekcije i kapsularne kontrakture. **Rezultati.** Među 726 bolesnica prosečne starosti 28,5 (22–48) godina, uvećanje dojki je učinjeno silikonskim implantima kroz inframamarni pristup koristeći submuskularnu i subglandularnu tehniku. Srednja vrednost implanta iznosila je 339 (200–520) cc. Subglandularna tehnika je primenjena kod 545 (75%), dok je kod 181 (25%) bolesnice implant ugrađen u submuskularni sloj: kompletno ispod mišića kod 95/726 (13%) odnosno „*dual plane*" tehnikom kod 86/726 (12%) bolesnica. U ranom postoeperativnom periodu nije bilo infekcije, zabeleženo je pet (0,7%) seroma i 8 (1,1%) hematoma, od kojih je 5 zahtevalo hiruršku reviziju. Nije bilo statistički značajne razlike u učestalosti komplikacija između navedenih tehnika. Tokom perioda praćenja zabeležene su tri (0,4%) kapsularne kontrakture. **Zaključak.** Učestalost komplikacija u ovoj grupi bolesnica nakon operacija uvećanja dojki bez korišćenja drenaže je mala. Buduće randomizirane studije su potrebne da potvrde uticaj drenaže rane na učestalost ranih i kasnih komplikacija.

Ključne reči:
mamoplastika; dojka, implantati; drenaža; postoperativne komplikacije.

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Introduction

The first successful breast augmentation was done in 1895 by Vincent Czerny, who transplanted a lipoma from the trunk to the breast in a patient deformed by a partial mastectomy [1]. The idea of breast augmentation was born. During 1950s and 1960s, a large number of different solid and semisolid alloplastic materials, like polyurethane, polytetrafluoroethylene (Teflon), expanded polyvinyl alcohol formaldehyde (Ivalon sponge), were injected into the breast parenchyma for the same purpose.

After the patients developed local tissue reaction, the use of these materials was discontinued [2]. In 1963, Cronin and Gerow [3] developed first modern silicone implant, using silicone gel as the filling material contained within a thin and smooth silicone elastomer shell. Since that time, breast augmentation has been one of the most popular aesthetic procedures. According to the latest International Society of Aesthetic Plastic Surgery (ISAPS) Global Survey, it makes 17% of all cosmetic procedures. There are several different approaches for breast augmentation. However, complications (implant related) of all these techniques are mainly common and they can be divided into early (within days or weeks of implantation) and those that typically occur "late" (months, years, or even decades later). Early complications, like infection, seroma, hematoma and capsular contracture, like some of the most frequent long-term complications, might be related to wound drainage [4–6]. According to some publications, these complications can be prevented by using wound drains [7]. Other authors claim that with the appropriate surgical technique, there is no need for drains because the use of drains is associated with a fivefold increased risk of infection [8]. Finally, in the latest Cochrane review from March 2013, no benefit from drainage in breast infections reduction was proved, but still, there was insufficient data for conclusion related to breast augmentation [9].

The aim of the study was to investigate the rate of complications in breast augmentations performed without drainage.

Methods

This is a retrospective analysis of 726 female patients who underwent cosmetic breast augmentation by a single surgical team, between 2003 and June 2013.

Demographic characteristics (age, body mass and height), surgical technique and complications (hematoma, infection, seroma and capsular contracture) data were taken from patients medical history charts. The augmentation surgery was carried out under general anesthesia with an overnight regimen. Patients were discharged home with a five days prescription of oral antibiotics and analgesics. Follow-up was performed at discharge, seven days and three months after the operation and yearly thereafter.

Surgical technique

Subglandular, complete submuscular and dual plane implant insertions were performed. All of them were done with 5 cm long inframammary incision. A pocket was created *via* electrocautery, scissors and finger dissection. In the dual plane technique, dissection in the retromammary plane was done approximately to the inferior border of the areola (type II) and to the superior border of the areola (type III). We stoped muscle division medially where the inframammary fold meets the sternum and medially, along the sternum, only the isolated, white, tendinous origins that lie laterally to the main body of the pectoralis were divided [10]. After hemostasis control, the implant pocket was irrigated with saline and on antibiotic. Silicon filled, textured, Cohesive I, round and anatomical implants were used. Both sides of the wound, were closed in three layers. All the augmentations were done without wound drainage. Perioperativly, all the patients received intravenous antibiotic prophylaxis. Immediately after the operation, when the patient was still at the surgical table, special type of bandaging with plaster (Sensifix®) was performed (Figure 1).

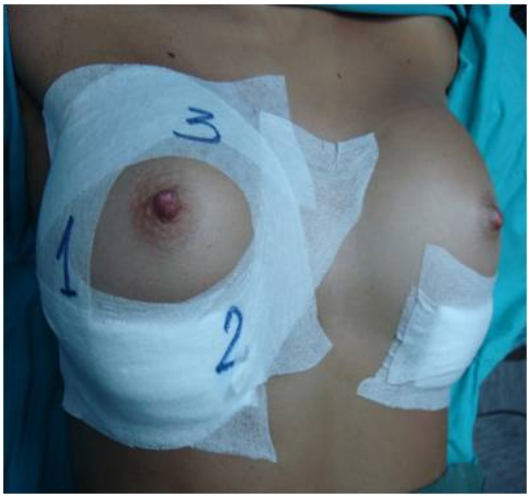

Fig. 1 – Postoperative bandaging.

Data analysis was performed using SPSS Software 11 (SPSS Inc, Chicago, Ill). All data are expressed as mean and standard deviation (SD). *t*-test and χ^2-test were used for parametric and nonparametric distributed values. p value < 0.05 was considered statistically significant.

Results

There were 726 patients of the average age of 28.5 (22–48) years. The average height and body mass were 171.44 (158–178) cm and 58 (46–75) kg, respectively. The average implant size was 339 (200–520) cc. The distribution of different types of implants in patients is presented in Table 1.

Table 1
Distribution of different implants in patients subjected to breast augmentation

Type of implant	Number	Percentage (%)
Mentor	440	61.1
Allergan	246	34.1
Polytech	35	4.8
Total	721	100

Subglandular augmentation had 545 (75%), while 181 (25%) of the patient received an implant in submuscular plane: dual plane technique was used in 86/726 (12%) and complete submuscular technique in 95/726 (13%) of the patients.

In early postoperative period, no infection was recorded. There were five (0.7%) seromas and eight (1.1%) hematoma, while five of them required surgical evacuation (Figure 2).

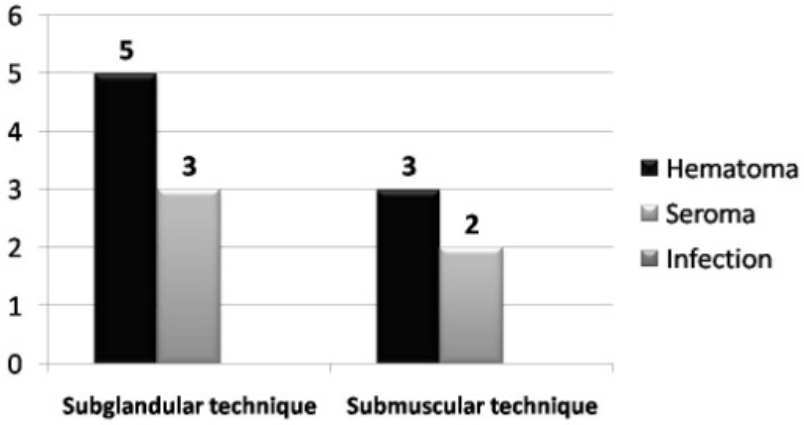

Fig. 2 – Early postoperative complications in patients subjected to breast augmentation procedures without drainage.

Using χ^2-test, there was no statistically significant difference among surgical techniques used in terms of complication rate.

During follow-up, there were three capsular contractures (0.4%). One capsular contracture was formed eleven months after the operation in a patient with small hematoma in early postoperative period which was treated conservatively. One was formed two years, and the third one three years after the operation.

Disscusion

When you ask your colleagues about how many of them are for using wound drainage in cosmetic breast augmentation, and how many are against, you will get different rate of answers. This is probably because there are many reasonable explainations for each of them.

Seroma is a rare postoperative complication and has unclear aetiology. Any cause that can enhance fluid exudation, could play the role in seroma formation. Fluid can lead to the loss of adhesion between implant and tissue with rotation of anatomical implant [11] and possible double capsula formation. Wound drainage clears away the fluid, so it can prevent these complications. But it is more likely that, late seroma and double capsula are caused, according to mechanical theory, when the adherence of the capsula to the implant is traumatically separated. The adherence can be seen in aggressively textured implant. The problem does not happen in the polyurethane implants because there was true tissue in growth that could not be separated from the implant [12]. We had five seromas, out of which three were submitted to needle aspiration, but none of these patients had formed any kind of capsular contracture.

According to some authors a positive correlation between hematoma and capsular contracture is about 86% [13], and the average rate of formation is 3–10.3% [14]. Hematoma seems not only to significantly increase the rate of capsular contraction but it also affects the time course, as contraction occurs more rapidly in the presence of hematoma [13]. In our group of eight hematomas, only one patient with hematoma that was not surgically evacuated, developed early capsular contracture. If all of these assumptions are true, what can we do to prevent them? Meticulous hemostasis for shore, but do we need drains? The absence of drainage could force the surgeon to pay more attention to hemostasis. Negative pressure in wound formed *via* drainage can slow coagulation process. The use of drains in breast augmentation is not only unnecessary but even deleterious.

The use of drains is associated with an increased risk of infection [15] and a large body of clinical data showing low capsular contracture rates when a drain is not used [11, 16–18]. In some findings, the length of time that a drain is left in the wound is in correlations with infection rate. According to some authors, the safe time is 12–18 h [7]. Systemic and especially local bacterial prophylaxis could control contamination [6]. We can totally agree with the latest reference because we used local antibacterial solutions and had no infections.

The disadvantage of our study is that it is retrospective one and without the control group.

Conclusion

The rate of complications in our group of patients submitted to breast augmentations is low, even no drainage at all. Randomized trials are needed to prove the role of drainage in prevention of complications after breast augmentation.

R E F E R E N C E S

1. *Czerny V*. Plastic replacement of the breast with a lipoma. Chir Kong Verhandl 1895; 2: 216. (German)
2. *Institute of Medicine*. Safety of Silicone Breast Implants. Washington, DC: National Academy Press; 1999.
3. *Cronin TD, Gerow FJ*. Augmentation mammaplasty: a new "natural feel" prosthesis. In: *Broadbent TR, American Association of Plastic Surgeons, American Society of Plastic and Reconstructive Surgeons, International Confederation for Plastic Surgery,* editors. Transactions of the Third International Congress of Plastic Surgery; 1963 October 13−18, Washington, DC, U.S.A. Amsterdam: Excerpta Medica Foundation; 1963. p. 41−9.
4. *Biggs T, Yarish RS*. Augmentation mammaplasty: a comparative analysis. Plast Reconstr Surg 1990; 85(3): 368−72.
5. *Gutowski KA, Mesna GT, Cunningham BL*. Saline-filled breast implants: a plastic surgery educational foundation multicenter outcomes study. Plast Reconstr Surg 1997; 100(4): 1019−27.
6. *Burkhardt BR, Dempsey PD, Schnur PL, Tofield JJ*. Capsular Contracture: a prospective study of the effect of local antibacterial agents. Plast Reconstr Surg 1986; 77(6): 919−32.
7. *Gherardini G, Zaccheddu R, Milner SM, El-Shazlyl M, Liapakis I*. Breast augmentation with silicone implants: the role of surgical drainage—report on 502 consecutive patients. Eur J Plast Surg 2006; 29(1): 9–12
8. *Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K*. Infections of breast implants in aesthetic breast augmentations: a single-center review of 3,002 patients. Aesthetic Plast Surg 2007; 31(4): 325−9.
9. *Stojkovic CA, Smeulders MJ, Van der Horst CM, Khan SM*. Wound drainage after plastic and reconstructive surgery of the breast. Cochrane Database Syst Rev 2013; 3: CD007258.
10. *Tebbetts JB*. Dual plane breast augmentation: optimizing implant-soft-tissue relationships in a wide range of breast types. Plast Reconstr Surg 2001; 107(5): 1255−72.
11. *Hedén P, Jernbeck J, Hober M*. Breast augmentation with anatomical cohesive gel implants. The world's largest current experience. Clin Plast Surg 2001; 28(3): 531−52.
12. *Elizabeth J*. Hall-Findlay. Breast Implant Complication Review: Double Capsules and Late Seromas. Plast Reconstr Surg 2011; 127(1): 56−66.
13. *Handel N, Jensen JA, Black Q, Waisman JR, Silverstain MJ*. The fate of breast implants: a critical analysis of complications and outcomes. Plast Reconstr Surg 1995; 96(7):1521−33.
14. *Brickman M, Parsa NN, Parsa FD*. Late hematoma after breast implantation. Aesthetic Plast Surg 2004; 28(2): 80−2.
15. *Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K*. Infections of breast implants in aesthetic breast augmentations: a single-center review of 3,002 patients. Aesthetic Plast Surg 2007; 31(4): 325−9.
16. *Adams WP Jr, Rios JL, Smith SJ*. Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. Plast Reconstr Surg 2006;117(1): 30−6.
17. *Chang L, Caldwell E, Reading G, Wray RC Jr*. A comparison of conventional and low-bleed implants in augmentation mammaplasty. Plast Reconstr Surg 1992; 89(1): 79−82.
18. *Bengtson BP, Van Natta BW, Murphy DK, Slicton A, Maxwell GP;* Style 410 U.S. Core clinical Study Group. Style 410 highly cohesive silicone breast implant core study results at 3 years. Plast Reconstr Surg 2007; 120(7 Suppl 1): 40S−8S.

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Treatment of sudden sensorineural hearing loss with hyperbaric oxygenation – our experience

Lečenje iznenadne senzorineuralne nagluvosti hiperbaričnom oksigenacijom – naša iskustva

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Abstract

Background/Aim. Sudden sensorineural hearing loss is manifested by the loss of hearing for more than 30 dB at three consecutive frequencies in the timeframe of 72 h. It is of unclear etiology and pathogenesis, which leads to the use of different therapeutic methods. Treatment protocols are not compliant, making it difficult to objectively quantify their impact. The aim of this study was to show the effect of hyperbaric oxygen therapy as the only method for the treatment of sudden sensorineural hearing loss. **Methods.** This retrospective study included 20 patients treated for sudden sensorineural hearing loss with hyperbaric oxygenation (HBO) in the period from 2004 to 2014. The study was conducted in the specialized medical clinic for hyperbaric and underwater medicine, HBO Medical Center in Belgrade. The patients were treated according to the following protocol: a session of 60 min at the pressure of 2 bars (2ATA) two times a day, a total of 30 sessions. Assessment of the therapy effects was performed by observing the change in the hearing threshold at the frequencies of 500, 1,000, 2,000 and 4,000 Hz at the end of the treatment. **Results.** After the completion of treatment according to the protocol, a full hearing recovery (total improvement of hearing damage or achieving final hearing threshold above 25 dB) was found in 11 (55%) of the patients. A partial recovery (hearing improvement of up to 15 dB, and a final hearing threshold below 45 dB) occurred in 4 (20%) of the patients. In 5 (25%) of the patients, improvement was not verified (there was no recovery or it was less than 5 dB). The average absolute hearing recovery was 24.94 dB. The mean relative hearing recovery was 65.45%. **Conclusion.** Because of the unclear multifactorial etiopathogenesis of this disease, there are many therapeutic protocols. Based on the results of our study HBO therapy could be recommended primarily as the treatment for sudden sensorineural hearing loss.

Key words:
hearing loss, sudden; hearing tests; hyperbaric oxygenation; recovery of function.

Apstrakt

Uvod/Cilj. Iznenadna senzorineuralna nagluvost manifestuje se gubitkom sluha za više od 30 dB na tri uzastopne frekvencije tokom 72 časa. Nejasne je etiologije i patogeneze, što je dovelo do upotrebe različitih terapijskih sredstava. Protokoli lečenja nisu usaglašeni, što otežava objektivnu kvantifikaciju njihovog učinka. Cilj rada bio je da se prikaže efekat terapije hiperbaričnom oksigenacijom (HBO) kao jedine metode u lečenju iznenadne senzorineuralne nagluvosti. **Metode.** Ova retrospektivna studija obuhvatila je 20 bolesnika lečenih od iznenadne senzorineuralne nagluvosti primenom hiperbarične oksigenacije u periodu od 2004. do 2014. godine u specijalističkoj lekarskoj ordinaciji za hiperbaričnu i podvodnu medicinu (HBO Medicinski centar u Beogradu). Lečenje je sprovedeno prema protokolu, 2 × dnevno po 60 min na pritisku od 2 bara (2 ATA), ukupno po 30 seansi. Procena dejstva primenjene terapije vršena je posmatranjem promene praga sluha na frekvencijama 500, 1 000, 2 000 i 4 000 Hz posle sprovedene kompletne terapije. **Rezultati.** Posle sprovedenog lečenja prema protokolu, potpuni oporavak sluha (postignuto potpuno poboljšanje oštećenja sluha, odnosno konačni prag sluha iznad 25 dB) zabeležen je kod 11 (55%) bolesnika. Delimičan oporavak (poboljšanje sluha do 15 dB, odnosno konačni prag sluha ispod 45 dB) zabeležen je kod 4 (20%)

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bolesnika. Kod 5 (25%) bolesnika nije potvrđeno poboljšanje (nije došlo do oporavka ili je ono bilo manje od 5 dB). Prosečan apsolutni oporavak sluha iznosio je 24,94 dB. Prosečni relativni oporavak sluha iznosio je 65,45%. **Zaključak.** Zbog nejasne multifaktorijalne etiopatogeneze ovog oboljenja postoji mnogo terapijskih protokola. Na osnovu rezultata našeg istraživanja može se preporučiti primarno lečenje iznenadne senzorineuralne nagluvosti hiperbaričnom oksigenacijom.

Ključne reči:
gluvoća, iznenadna; sluh, ispitivanje; hiperbarična oksigenacija; funkcija, povratak.

Introduction

Sudden sensorineural hearing loss (SSHL) is the sudden total or partial hearing loss of sensorineural type, often without clear cause. The literature describes it under various names: acute hearing loss, acute sensorineural hearing loss, and acute cochlear deafness. The disease was described for the first time by De Kleyn about 60 years ago. It occurs spontaneously within a few seconds, minutes or hours, it is mostly one-sided. The average hearing loss is between 50 and 70 dB. The American Society of Otorhinolaryngologists (American Academy of Otorhinolaryngology) defines the sudden hearing loss as a kind of sensoneural hearing loss with a reduction in hearing threshold of at least 30 dB at three consecutive frequencies, over a period of 72 hours [1]. It is characterized by buzzing, and sometimes dizziness of varying intensity [2]. It is rarely seen in children and usually affects the population aged 20 to 60 years of age and it is equally represented among the sexes [3]. It can occur in people with previously normal hearing or as an acute deficit of the previously damaged hearing. It is usually unilateral, but it occurs in both sides with the same frequency. In about 2% of the cases it affects both sides at the same time [4]. The incidence is 5 to 20 patients per 100,000 population per year 5. The etiology most often cannot be established. In the literature, there are more than 100 causal agents for the disease quoted [5] and the most frequently referred are viral infections, circulatory disorder, trauma, autoimmune disease of the inner ear. However, the most common form is the idiopathic sudden sensorineural hearing loss and it is present in about 80%, and according to some authors up to 90% of the cases of sudden hearing loss [6]. According to the latest theories on the etiology of sudden sensorineural hearing loss, the following factors are of importance: the anatomy of the blood vessels of the inner ear, physiology of microcirculation of blood and inner ear, cell metabolism of Corty`s organ [7]. Because of multiple etiological explanations for the genesis of the disease in the last 50 years, 50 different treatment protocols have been developed [5]. They range from the use of vasoactive drugs, corticosteroids, hyperbaric oxygenation (HBO), vitamin E, sedatives, calcium antagonists, prostaglandins, carbogen, magnesium, preparation of Ginkgo biloba extract, to surgical procedures. The greatest significance for a favorable outcome of the treatment has immediate implementation of therapy and the implementation of the entire treatment protocol. After the application of adequate therapy a good recovery is often achieved, although some patients have spontaneous and complete recovery of hearing without any treatment. Relapses are also possible [8].

Hyperbaric medicine is a clinical discipline where the main therapeutic substrate is 100% oxygen (O_2) that is inhaled under conditions of high pressure, above 1 bar (101 kPa, 760 mmHg), also known as hyperbaric oxygen, and is applied in special devices, hyperbaric chambers [9]. HBO therapy as a therapeutic method has been used for the treatment of various diseases in the field of human medicine, and for the treatment of sudden hearing loss as well.

HBO increases the amount of oxygen in the tissues at the expense of physically dissolved oxygen in the blood plasma and thus alleviates or completely eliminates the hypoxia. It also improves blood circulation by reducing plasma viscosity, reducing platelet aggregation, accelerating neocapilarization, the creation of new blood vessels and increasing the flexibility of red blood cells [9].

The aim of this study was to investigate the effect of HBO therapy as the single method for the treatment of sudden sensorineural hearing loss.

Methods

This retrospective study included 20 patients with unilateral sudden sensorineural hearing loss, treated with hyperbaric oxygen in the period from 2004 to 2014 in a specialized medical clinic for hyperbaric and underwater medicine, HBO Medical Center in Belgrade. The symptoms occurred for the first time in all of the subjects, what was verified by medical history of each patient.

The diagnosis of sudden sensorineural hearing loss in all the patients was made based on anamnesis, otorhinolaryngology examination, audiometry, impedancemetry and in case of present dizziness, vestibular testing was done. The existence of previous hearing loss, tinnitus and vertigo were determined by anamnestical data. In all the patients unilateral sensorineural hearing loss of varying degrees was confirmed on the basis of pure tone audiometry.

The study included only patients with sudden hearing loss, with hearing decrease of more than 30 dB at three consecutive frequencies in the range between 500 Hz and 4000 Hz in 72 h. The patients were divided according to gender, age, the presence of other symptoms (tinnitus, vertigo) and shape of audiometric curve.

On the basis of hearing loss (average hearing loss at frequencies 500, 1,000, 2,000 and 4,000 Hz), all the subjects were divided into 4 groups. The first group consisted of subjects with mild hearing loss (40 dB), the other with moderate hearing loss (41 dB to 60 dB), the third with a severe hearing loss (61 dB to 80 dB), and the fourth group of patients with deafness (over 80 dB).

The treatment was done according to the following protocol: a session of 60 min at the pressure of 2 bars (2ATA) twice a day, in total 30 sessions over 15 days. The result of treatment was assessed on the basis of changes in the average hearing threshold at frequencies 500, 1,000, 2,000 and 4,000 Hz after the end of treatment. Based on the results obtained, the treated patients were divided into 3 groups. The group I included patients with no recovery recorded, or it was less than 5 dB. The group II included patients with recorded hearing improvement to 15 dB and whose final hearing threshold was below 45 dB. In the group III there were patients who achieved complete improvement of the quality of hearing, or those with the final hearing threshold above 25 dB. Hearing threshold of the affected ear before the treatment was marked as the initial hearing threshold (IHT). ITH is equal to the average hearing threshold at 500, 1,000, 2,000 and 4,000 Hz of the affected ear. In the same way the final hearing threshold (FHT) was determined, which was equal to the average hearing threshold at 500, 1,000, 2,000 and 4,000 Hz of the affected ear at the end of the therapy. Improvement of hearing we defined as absolute and relative. Absolute hearing improvement (AHI) was the difference in decibels between the initial and final hearing threshold (AHI = IHT - FHT). Relative hearing improvement (RHI) was the quotient of absolute hearing improvement, and the differences between the initial hearing threshold of the affected ear and the average hearing threshold (AHT) of the second a healthy ear, multiplied with 100 (RHI = AHI / IHT – AHT x 100). (AHT = average hearing thresholds at 500, 1,000, 2,000, 4,000 Hz).

Results

The study included 13 (65%) men and 7 (35%) women. There was no statistically significant difference in the distribution of sexes (= 1.8; df = 1, p > 0.05). The average age of the patients was 48.5 ± 14.37 years; the youngest patient was 21 and the oldest 75 years. The distribution of the age variable was normal, which allowed the use of parametric tests. Analysis of age in relation to gender showed no statistically significant difference in age between male and female (t = 0.048, df = 18; p > 0.05).

In all the patients unilateral hearing loss was present. Analyzing body sides, no statistically significant difference was recorded (= 0.2; df = 1; p > 0.05), the right-sided hearing loss was diagnosed in 11 (55%) and sinistral in 9 (45%) patients.

In addition to hearing loss, we observed other symptoms (Table 1). The most common symptom was second tinnitus, present in 9 (45%) patients. Vertigo was present in 7 (35%). Without hearing impairment in addition to other symptoms there were 4 patients (20%).

Distribution of respondents according to the degree of hearing impairment is shown in Table 1. There was no statistically significant difference in the degree of hearing impairment in the analyzed sample (= 6.8, df = 3; p > 0.05).

Distribution of respondents according to the shape of audiometric curves is given in Table 1. For most respondents, 15 (75%), audiometric curve was porcine descending type, and $\chi 2$ test showed that the difference in the number of patients due to the type of curves was statistically highly significant (= 26.8; df = 3; p < 0.01) in favor of a downward curve.

Upon completion of the entire treatment protocol with HBO therapy 11 (55%) patients had a complete recovery of hearing (final hearing threshold above 25 dB). A partial recovery (hearing aids of up to 15 dB and a final hearing threshold below 45 dB) was achieved in 4 (20%) patients. There was no hearing improvement or it was less than 5 dB in 5 (25%) patients, of which one respondent (5%) had a complete loss of hearing.

The average ITH of all the patients at all frequencies (500; 1,000, 2,000 and 4,000 Hz) was 60.04 dB, while the average FTH was 35.10 dB (Table 2).

The average absolute hearing recovery of all the patients at all frequencies amounted to 24.94 dB. The average relative improvement in hearing of all the patients at all frequencies amounted to 65.45% (Table 3).

Table 1

Clinical characteristics of patients with sudden sensorineural hearing loss

Characteristics	Number (%) of patients
Symptoms	
partial hearing loss	20 (100)
tinitus	9 (45)
vertigo	7 (35)
none	4 (20)
Degree of hearing impairment	
easy (< 40 dB)	4 (20)
mild (41–60 dB)	9 (45)
hard (61–80 dB)	6 (30)
deftness (> 80 dB)	1 (5)
Shape of audiometric curve	
horizontal	2 (10)
descending	15 (75)
recessed	2 (10)
deftness curve	1 (5)

Table 2

Hearing threshold prior and after the therapy

Hearing threshold	500 Hz	*p*	1000 Hz	*p*	2000 Hz	*p*	4000 Hz	*p*	Average	*p*
Initial (dB)	33.25	0.000	58.75	0.000	69.47	0.000	78.68	0.000	60.04	0.000
Final (dB)	22.75		34.75		39.74		43.16		35.10	

Table 3

Hearing improvement after the therapy

Hearing improvement	500 Hz	1000 Hz	2000 Hz	4000 Hz	Average
Absolute (dB)	10.50	24.00	29.73	35.52	24.94
Relative (dB)	79.25	61.94	60.10	60.53	65.45

T-test for related samples demonstrated a highly statistically significant difference ($p < 0.001$) in the threshold of hearing on audiogram before and after oxygen therapy at four frequencies (500, 1,000, 2,000 and 4,000 Hz), which speaks to the fact that the application of HBO treatment of sudden hearing impairment gives good results.

Discussion

The etiology of sudden sensorineural hearing loss has not yet been fully tested. Experimental studies have shown that the most common pathological changes are found in their striatum vascularis (capillaries are longer and of narrower lumens, causing decreased blood stream at the threshold of hypoxia). The use of medications that increase the perfusion of the inner ear (the percentage of oxygen, glucose utilization) allows cells the organ of Corti to survive until the restoration of normal conditions for the function [7].

In this study, hearing improvement after the therapy was defined as a decrease in the threshold of the pure tone of 10 dB or more at four frequencies (500, 1,000, 2,000 and 4,000 Hz), regardless of the level of hearing loss at the beginning [1].

The Siegel classification is based on the significance of the initial hearing loss, as the recovery of, for example, 20 dB does not have the same significance when it occurs at the level between 25 and 40 dB, or at the level of over 45 dB [10].

According to the classification that was used in this study to assess the success of treatment with HBO, for the effectiveness of the therapy is used an average increase (in dB) at four speech audiometric frequencies (500, 1,000, 2,000 and 4,000 Hz) [1].

It was pointed out that the initial degree of hearing loss is very important prognostic indicator for the outcome of the treatment of sudden hearing loss [11].

Although the results of most studies suggest that therapy should begin as soon as possible [11, 12,] some authors believe that acute hearing loss is not a medical emergency and treatment is not necessary to start in the first 24 h, but in the first 7 days [13].

Comparing the two ways of treating patients with sudden deafness – vasoactive agents and corticosteroids, lead to the conclusion that the use of vasoactive agents offers clinically evident improvements, and corticosteroids a greater percentage of early recovery of hearing [14].

Also, comparison of the two ways of treating patients with sudden deafness (HBO and pentoxifylline), shows that in the group of patients treated with HBO hearing improvement was statistically significantly higher than in the group of patients treated with infusions of pentoxifylline [15].

Similarly, in a Pezzoli et al. [16] study hearing improvement was significantly greater in patients treated with HBO compared to the control group (patients not treated).

Comparison of the results of co-administration of HBO and drug therapy on the one hand, and the only drug therapy on the other, shows that significantly better results are achieved in patients treated with combination therapy than in those treated only with drug therapy [17].

Analysis of possible therapy protocols, after the cessation of the effect of treatment with systemic corticosteroids, shows that the application of HBO and intratimpanic instillation of corticosteroids can be life-saving in these patients [18].

Studies show that HBO addition to usual treatment significantly improves the outcome of idiopathic sudden sensorineural hearing loss [19].

Conclusion

Due to unclear multifactorial etiopathogenesis of sudden sensorineural hearing loss there are many therapeutic protocols. Further prospective clinical trials are expected to come to the conclusion which method of treatment or combination therapy protocols should be given priority. Based on the results of our study hyperbaric oxygen can be recommended as primary treatment of sudden sensorineural hearing loss.

REFERENCES

1. *Živić LJ, Živić Đ, Stojanović S*. Sudden hearing loss: Our experience in the treatment with vasoactive agents. Srp Arh Celok Lek 2008; 136(3−4): 91−4.
2. *Rambold H, Boenki J, Stritzke G, Wisst F, Neppert B, Helmchen C*. Differential vestibular dysfunction in sudden unilateral hearing loss. Neurology 2005; 64(1): 148−51.
3. *Shemirani NL, Schmidt M, Friedland DR*. Sudden sensorineural hearing loss: An evaluation of treatment and management approaches by referring physicians. Otolaryngol Head Neck Surg 2009; 140(1): 86−91.
4. *Xenellis J, Nikolopoulos TP, Stavroulaki P, Marangoudakis P, Androulakis M, Tsangaroulakis M,* et al. Simultaneous and sequential bilateral sudden sensorineural hearing loss: Are they different from unilateral sudden sensorineural hearing loss. ORL J Otorhinolaryngol Relat Spec 2007; 69(5): 306−10.
5. *Maslovara S*. The primary treatment of idiopathic sudden hearing loss with oxygen under high pressure [thesis]. Zagreb: Faculty of Medicine, University of Zagreb; 1999. (Croatian)
6. *Hughes GB, Freedman MA, Haberkamp TJ, Guay ME*. Sudden sensorineural hearing loss. Otolaryngol Clin North Am 1996; 29(3): 393−405.
7. *Mihailović-Kokić B, Todorović AJ, Kokić Z*. Acute deafness-personal experience with therapy. Srp Arh Celok Lek 2002; 130(11−12): 394−6. (Serbian)
8. *Furuhashi A, Matsuda K, Asahi K, Nakashima T*. Sudden deafness: Long-term follow-up and recurrence. Clin Otolaryngol Allied Sci 2002; 27(6): 458−63.
9. *Živković M*. Manual for Hyperbaric Medicine. Belgrade: Srpska zdravstvena organizacija; 2010. (Serbian)
10. *Siegel LG*. The treatment of idiopathic sudden sensorineural hearing loss. Otolaryngol Clin North Am 1975; 8(2): 467−73.
11. *Byl FM*. Sudden hearing loss: Eight years' experience and suggested prognostic table. Laryngoscope 1984; 94(5 Pt 1): 647−61.
12. *Shaia FT, Sheehy JL*. Sudden sensori-neural hearing impairment: A report of 1,220 cases. Laryngoscope 1976; 86(3): 389−98.
13. *Ćvorović LJ, Đerić D, Pavićević LJ, Probst R, Hegemann S*. Does Does acute sensorineural deafness befall to urgent conditions? Vojnosanit Pregl 2009; 66(1): 35−8. (Serbian)
14. *Živić LJ, Živić D, Đonović N*. Sudden hearing loss: Our experience in the treatment with vasoactive agents and corticosteroid drugs. Srp Arh Celok Lek 2012; 140(3−4): 153−8.
15. *Racic G, Maslovara S, Roje Z, Dogas Z, Tafra R*. Hyperbaric Oxygen in the Treatment of Sudden Hearing Loss. ORL J Otorhinolaryngol Relat Spec 2003; 65(6): 317−20.
16. *Pezzoli M, Magnano M, Maffi L, Pezzoli L, Marcato P, Orione M,* et al. Hyperbaric oxygen therapy as salvage treatment for sudden sensorineural hearing loss: a prospective controlled study. Eur Arch Otorhinolaryngol 2015; 272(7): 1659−66.
17. *Dundar K, Gumus T, Ay H, Yetiser S, Ertugrul E*. Effectiveness of hyperbaric oxygen on sudden sensorineural hearing loss: Prospective clinical research. J Otolaryngol 2007; 36(1): 32−7.
18. *Čvorović LJ, Jovanović MB, Milutinović Z, Arsović N, Djeric D*. Randomized prospective trial of hyperbaric oxygen therapy and intratympanic steroid injection as salvage treatment of sudden sensorineural hearing loss. Otol Neurotol 2013; 34(6): 1021−6.
19. *Topuz E, Yigit O, Cinar U, Seven H*. Should hyperbaric oxygen be added to treatment in idiopathic sudden sensorineural hearing loss. Eur Arc Otorhinolaryngol 2004; 261(7): 393−6.

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GENERAL REWIEV

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Atherosclerosis and coronary artery bifurcation lesions: anatomy and flow characteristics

Ateroskleroza račvi koronarnih arterija: anatomske i hemodinamske karakteristike

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Key words:
coronary vessels; coronary disease; atherosclerosis; angioplasty, baloon, coronary.

Ključne reči:
koronarni krvni sudovi; koronarna bolest; arterioskleroza; angioplastika, translumenska, perkutana, koronarna.

Introduction

Although the coronary arterial tree is uniformly exposed to the systemic risk factors, we have learned since the beginning of the 20th century that atherosclerotic "plaques" preferentially develop at vessel branch points [1–3]. As a result, coronary bifurcations in recent reports account for 15–20% of all percutaneous coronary interventions (PCI) [4–6]. Coronary artery bifurcations are predilection sites for atherosclerosis development as a result of specific flow characteristics and local endothelial shear stress (ESS) presentation [7]. Anatomic features of coronary bifurcations, such as diameter of the main vessel (MV) and the side branches (SB), atherosclerotic plaque burden in the proximal and distal part of the MV and the SB, and bifurcation angle, all impact on local flow patterns [8]. The relationship among the coronary anatomy, local flow and vascular biology promotes the progression and complexity of atherosclerosis in coronary bifurcations. The complex local hemodynamic microenvironment after bifurcation stenting also influences in-stent restenosis, thrombosis and clinical outcomes [9].

In this review, we summarized the current data with respect to coronary artery bifurcation anatomy and flow characteristics, the impact of local hemodynamic conditions in initiation and progression of atherosclerosis and application of this basic knowledge in contemporary PCI.

Fractal geometry of coronary artery bifurcations

Ramifications of the coronary tree follow the natural law of conservation of mass and minimum energy expenditure in providing underlying myocardium with the optimum amount of blood [8, 10]. According to the law of conservation of mass, the flow (Q) through the proximal, "mother" segment of the MV must equal the sum of the flow through the two "daughter" vessels (specifically, distal part of the MV and the SB, Figure 1) [8]. Since flow is related to the lumen cross-sectional area and flow velocity, there is a relation between the function (blood flow) and anatomy or geometry (cross-sectional diameter and area) [4, 6]. There are many theories of the vascular tree design based on the concept of minimum work. Murray's law (also known as the "cube law"), is based on a minimum energy hypothesis, and states that the sum of the cubes of the "daughter" vessel diameters ($D_{daughter1}$ and $D_{daughter2}$) is equal to the cube of the "mother" vessel diameter (D_{mother}) as : $D_{mother}^3=D_{daughter1}^3+D_{daughter2}^3$ (Figure 1) [8]. Finet et al. [8] show that Murray's law cannot be applied in the entire coronary tree and suggest a $^{7/3}$ exponent in their HK model: $D_{mother}^{7/3}=D_{daughter1}^{7/3}+D_{daughter2}^{7/3}$. Finally, Finet et al. [8] (Figure 1) proposed a linear relation ($D_{mother}=0.678*[D_{daughter1}+D_{daughter2}]$) based on regression analysis of Y-type bifurcation (where 0.678 expresses the ratio of the "mother" vessel diameter to the sum of two "daughter" vessel diameters) [8].

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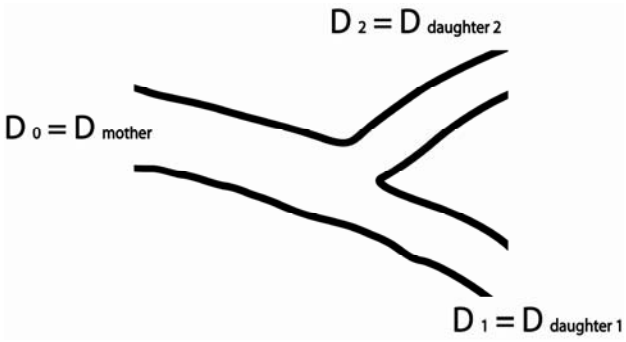

Fig. 1 – Fractal anatomy of coronary artery bifurcation scheme. The mother vessel divides into two daughter vessels and the diameter of the mother vessel (D_0) is greater than any of the two daughter vessel diameters (D_1 and D_2).

Therefore, coronary tree does not taper linearly and change in diameter occurs predominantly at bifurcation points. As a consequence, each bifurcation consists of three segments with different diameters which have the fractal geometry, with a constant relation between diameters defined by the scaling laws [5–8]. The proximal "mother" segment of the MV is consequently always larger in diameter compared to the distal segments (Figure 2), and equals (according to fractal ratio equation) approximately two thirds of the sum of two "daughter" vessels diameters [10]. This natural fractal law has to be kept in mind for optimal selection of material for PCI.

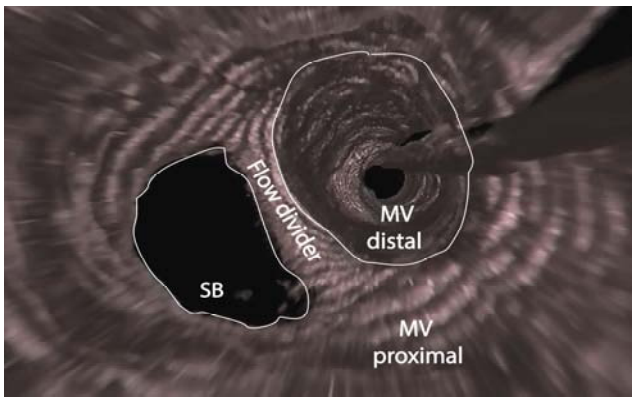

Fig. 2 – 3D optical coherence tomography pullback reconstruction, showing a larger proximal main vessel (MV) diameter and smaller distal MV and side branch (SB) diameters, separated by a flow divider.

Flow distribution in coronary bifurcations

ESS is the tangential force applied to the luminal endothelial layer, which is caused by the friction of blood flow and is expressed in units of force / unit area [2]. Arterial bifurcations are known for their characteristic flow velocity patterns resulting in flow separation, recirculation and secondary flow patterns leading to local low and oscillatory ESS along the lateral walls, while high ESS develops in the flow divider (carina) of the bifurcation (Figure 3) [7, 11–14]. The proportion of flow directed towards the SB determines the ESS patterns within the bifucration [2]. Computational flow dynamic studies acknowledged different patterns of flow and the impact of anatomic variations in the flow profile in coronary bifurcations [13, 15]. Furthermore, *in vitro* studies show that bifurcation angle and diameter also play an important role in local ESS patterns, and wider angles and larger vessel diameters create a greater turbulence in flow and consequently create lower ESS [16]. Finaly, the ratio between the MV and the SB diameters influences flow hemodynamic profile [17].

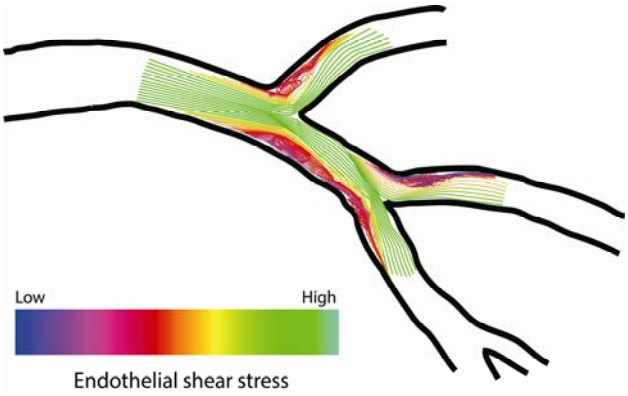

Fig. 3 –Endotelial shear stress distribution in coronary artery bifurcation illustrating low values at the lateral walls and high values at the carina.

Further complexity of hemodynamic profile is introduced by temporal alterations in ESS caused by flow pulsatility [18]. A typical temporal pattern is characterized by low and oscillatory ESS during heart systole and rapid increase and then slow decrease during diastole [18]. These temporal changes may also have an impact on the process of initiation and progression of atherosclerosis [7, 19].

Atherosclerotic plaque development and progression

In 1969 Caro et al. [20] first associated ESS with atherosclerotic plaque formation. Low or oscillatory ESS promotes plaque formation inside the entire vascular tree and there is a strong correlation between endothelial dysfunction, low shear stress and oscillatory flow, predominantly at sites of bifurcations and at curvatures [2, 13, 14, 21–23]. The mechanism of plaque creation in low ESS areas is very complex but several reports imply that low ESS modulates the molecular, cellular and vascular dynamics, which are responsible for atherogenesis and progression of plaque towards a high-risk phenotype [3, 24, 25]. Endothelial cells have important role because they alter gene expression pattern in response to flow-mediated ESS changes, with consequent activation of pro-atherogenic transcription factors [18]. Shear stress plays an important role not only in early plaque formation but also its progression. Plaque progression is initiated by endothelial dysfunction, with increased permeability for lipoproteins and with up-regulation of adhesion molecules, such as intercellular adhesion molecules 1, vascular cell adhesion molecules and leukocyte transmigration [26, 27].

Histopathology of atherosclerosis in coronary artery bifurcations

Pathologic studies showed that atherosclerosis predominantly involves the outer (lateral) wall of vessel bi-

furcations opposite to carina, which corresponds to low and oscillatory ESS regions (Figure 4) [28–30]. Autopsy studies also describe the presence of intimal thickening in the lateral wall of bifurcation, with the absence of lesion formation at the inner wall (flow divider or carina) [31]. In addition, atherosclerotic lesions were more frequent in autopsy specimens on the myocardial side than on the epicardial side of the arteries [31]. Pathologic studies in swine showed eccentric neointimal hyperplasia at the lateral wall following MV stenting, with concurrent acute adhesion and accumulation of leukocytes, while leukocytes were absent at the carina [17]. Nakazawa et al. [29] determined plaque distribution inside the MV and the SB in patients dying from sudden coronary death [29]. Each bifurcation was morphologically assessed including intimal thickness and necrotic core distribution in the MV (proximal and distal to SB origin), in the SB and at the carina region. Similar to prior reports, lateral walls showed a significantly greater intimal thickness as compared to those in the flow divider region [29]. Plaque thickness was highest at the lateral wall in the distal MV, followed by the lateral wall in the proximal MV. Similarly, advanced atherosclerotic plaques with necrotic core were also significantly greater at the lateral regions as compared with the flow divider area, where necrotic core formation was usually minimal or frequently absent. Interestingly, plaque progression might influence local geometry by increasing lumen obstruction with subsequent flow acceleration. This observation may explain plaque formation at high ESS areas in bifurcations [15].

Fig. 4 – Histologic image of coronary plaque in native bifurcation lesion. Longitudinal section shows necrotic core accompanied with calcification within the plaque predominantly at low endothelial shear stress (ESS) areas (lateral wall oposite to carina), whereas high ESS (flow divider/carina) has minimal intimal thickening.

Autopsy studies following bifurcation stenting showed increased rate of restenosis in patients treated with bare metal stents, a higher risk of late stent thrombosis with drug-eluting stents and similar risk of acute and subacute stent thrombosis (< 30 days) with both stent types [32]. Joner et al. [32] documented differences in the healing patterns in cases with drug-eluting stents as compared with bare metal stents. In cases with drug-eluting stents, delayed vascular healing, with uncovered struts and fibrin deposition were significantly greater at the carina sites compared to the lateral walls and most of the thrombi in these cases originate at the carina sites [32]. It has been demonstrated that low ESS upregulates growth factors and increases local thrombogenicity while high ESS augments platelet activation and aggregation [2, 33].

Importantly, stent design characteristics and the relationship between stent struts and vessel wall influence flow hemodynamics and clinical outcomes [34]. Stents with thicker struts and less streamlined configuration (*ie* rectangular configuration) have higher rates of in-stent restenosis, likely due to the generation of high ESS proximal and above the stent struts and low ESS with flow recirculation downstream (distal) to the struts [2, 9, 35]. Stent overlap aggravates local flow hemodynamics which may have impact on clinical outcomes [36].

Clinical implications

Percutaneous treatment of bifurcation lesion remains technically challenging and still results in higher rates of procedural complications [4]. Based on randomized clinical trials provisional SB stenting is the recommended approach for the majority of bifurcation lesions and with this approach, a single stent can be used in 80–90% of cases with optimal clinical outcome [4, 6]. Application of information on fundamental aspects of coronary bifurcation anatomy and flow characteristics is essential to fully understand the technical approach used for the provisional SB stenting strategy. Procedural planning starts with the analysis of coronary angiography and anticipation of plaque distribution in accordance with the knowledge acquired from pathologic studies (Figures 5A and 5B).

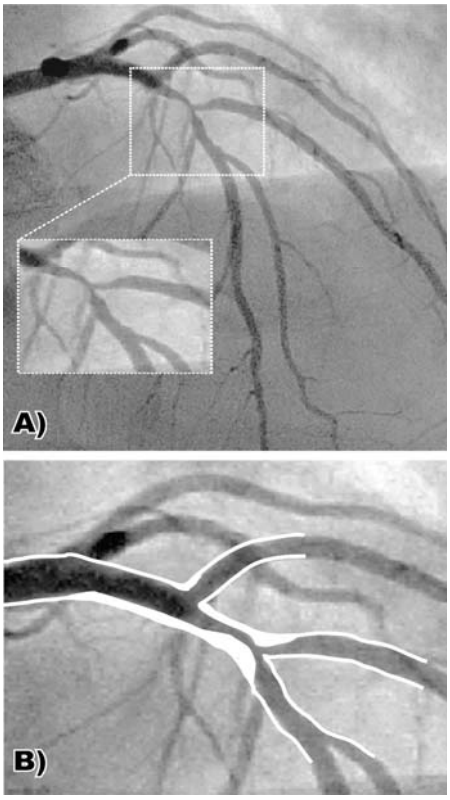

Fig. 5 – A) Coronary angiography of true bifurcation lesion, with significant disease of the main vessel (left anterior descending) and the side branch (diagonal branch); B) Schematic reconstruction of vessel wall and lumen contours, with atherosclerotic plaque area depicted in white.

The procedure begins with MV stenting and, keeping in mind that every bifurcation consists of three segments with different diameters, selection of stent diameter for the MV is crucial. If stent diameter is selected according to the proximal MV reference diameter, it will be oversized for the distal MV and may therefore increase the risk of SB occlusion created by carina shifting [4–6]. Therefore, MV stent diameter should be selected fitting to the distal MV diameter, with understanding that struts will not be opposed in the proximal MV (Figure 6A) [4–6]. Proximal optimization technique is proposed to correct MV stent malapposition by inflating a short bigger balloon and positioning of distal marker at carina (Figure 6B). Residual stenosis in the SB can be treated with different stent implantation techniques, like stenting and small protrusion, internal mini-crush or *culotte*. *Culotte* stenting is characterized by second stent implantation with the overlap in the proximal MV (Figure 7A). The procedure is finalized by the final kissing balloon inflation (Figure 7B). Therefore, application of basic knowledge from pathology and flow analysis allows adjustment of metallic stents to natural fractal anatomy and geometry of bifurcation lesions, especially in bifurcations with large SB, because of a larger difference in the diameters between the proximal and the distal part of the MV.

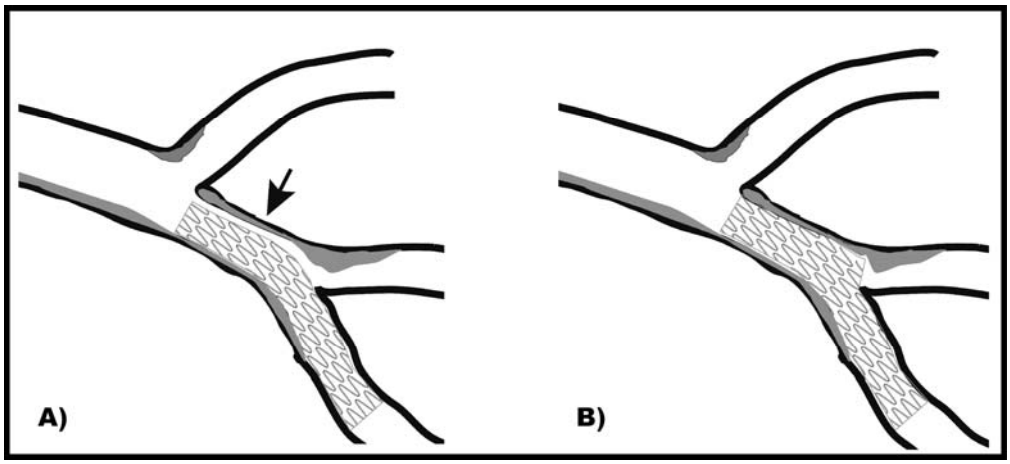

Fig. 6 – A) Schematic presentation of main vessel (MV) stenting, with stent malapposition in the proximal segment of the MV (arrow); B) optimal stent apposition following proximal optimization technique.

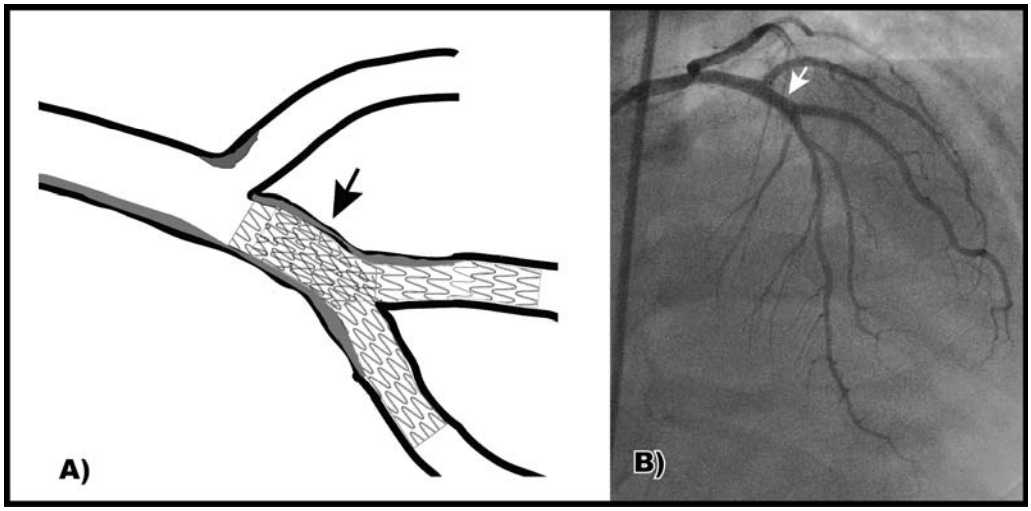

Fig. 7 – A) Schematic presentation of *culotte* stenting technique in lefi anterior descending /diagonal bifurcation, with proximal overlap of the two stents (black arrow); B) final angiographic result following kissing balloon inflation (white arrow).

Conclusion

Coronary bifurcations lesions have particular anatomic and hemodynamic characteristics, which influence the selection of appropriate percutaneous coronary intervention strategy. A complex interaction between the coronary anatomy, local flow characteristics and vascular biology influences development and progression of atherosclerosis in coronary bifurcations. Anticipation of plaque distribution at bifurcation site and application of knowledge from computational fluid dynamic studies allow accurate selection and application of percutaneous coronary intervention strategies.

R E F E R E N C E S

1. *Fry DL*. Certain histological and chemical responses of the vascular interface to acutely induced mechanical stress in the aorta of the dog. Circ Res 1969; 24(1): 93−108.
2. *Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH*. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J Am Coll Cardiol 2007; 49(25): 2379−93.
3. *Chatzizisis YS, Jonas M, Coskun AU, Beigel R, Stone BV, Maynard C*, et al. Prediction of the localization of high-risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: An intravascular ultrasound and histopathology natural history study. Circulation 2008; 117(8): 993−1002.
4. *Lassen JF, Holm NR, Stankovic G, Lefevre T, Chieffo A, Hildick-Smith D,* et al. Percutaneous coronary intervention for coronary bifurcation disease: Consensus from the first 10 years of the European Bifurcation Club meetings. EuroIntervention 2014; 105: 545−60.
5. *Hildick-Smith D, Lassen JF, Albiero R, Lefevre T, Darremont O, Pan M*, et al. Consensus from the 5th European Bifurcation Club meeting. EuroIntervention 2010; 6(1): 34−8.
6. *Stankovic G, Lefevre T, Chieffo A, Hildick-Smith D, Lassen JF, Pan M*, et al. Consensus from the 7th European Bifurcation Club meeting. EuroIntervention 2013; 9(1): 36−45.
7. *Soulis JV, Giannoglou GD, Chatzizisis YS, Farmakis TM, Giannakoulas GA, Parcharidis GE*, et al. Spatial and phasic oscillation of non-Newtonian wall shear stress in human left coronary artery bifurcation: an insight to atherogenesis. Coron Artery Dis 2006; 17(4): 351−8.
8. *Finet G, Huo Y, Rioufol G, Ohayon J, Guerin P, Kassab GS*. Structure-function relation in the coronary artery tree: From fluid dynamics to arterial bifurcations. EuroIntervention 2010; 6 Suppl J: J10−5.
9. *Van der Heiden K, Gijsen FJ, Narracott A, Hsiao S, Halliday I, Gunn J,* et al. The effects of stenting on shear stress: Relevance to endothelial injury and repair. Cardiovasc Res 2013; 99(2): 269−75.
10. *Colombo A, Stankovic G*. Bifurcations and branch vessel stenting. In: *Topol EJ, Teirstein PS,* editors. Textbook of interventional cardiology. 6th ed. Philadelphia: Saunders Elsevier; 2012. p. 270−87.
11. *Giannoglou GD, Soulis JV, Farmakis TM, Giannakoulas GA, Parcharidis GE, Louridas GE*. Wall pressure gradient in normal left coronary artery tree. Med Eng Phys 2005; 27(6): 455−64.
12. *Soulis J, Fytanidis D, Seralidou K, Giannoglou G*. Wall shear stress oscillation and its gradient in the normal left coronary artery tree bifurcations. Hippokratia 2014; 18(1): 12−6.
13. *Blagojevic M, Nikolic A, Zivkovic M, Zivkovic M, Stankovic G*. Influence of blocks' topologies on endothelial shear stress observed in CFD analysis of artery bifurcation. Acta Bioeng Biomech 2013; 15(1): 97−104.
14. *Blagojevic M, Nikolic A, Zivkovic M, Zivkovic M, Stankovic G*. A novel framework for fluid/structure interaction in rapid subject-specific simulations of blood flow in coronary artery bifurcations. Vojnosanit Pregl 2014; 71(3): 285−92.
15. *Gijsen FJ, Schuurbiers JC, van de Giessen AG, Schaap M, van der Steen AF, Wentzel JJ*. 3D reconstruction techniques of human coronary bifurcations for shear stress computations. J Biomech 2014; 47(1): 39−43.
16. *Huo Y, Finet G, Lefevre T, Louvard Y, Moussa I, Kassab GS*. Which diameter and angle rule provides optimal flow patterns in a coronary bifurcation. J Biomech 2012; 45(7): 1273−9.
17. *Richter Y, Groothuis A, Seifert P, Edelman ER*. Dynamic flow alterations dictate leukocyte adhesion and response to endovascular interventions. J Clin Invest 2004; 113(11): 1607−14.
18. *Wentzel JJ, Chatzizisis YS, Gijsen FJ, Giannoglou GD, Feldman CL, Stone PH*. Endothelial shear stress in the evolution of coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions. Cardiovasc Res 2012; 96(2): 234−43.
19. *Giannoglou GD, Chatzizisis YS, Zamboulis C, Parcharidis GE, Mikhailidis DP, Louridas GE*. Elevated heart rate and atherosclerosis: An overview of the pathogenetic mechanisms. Int J Cardiol 2008; 126(3): 302−12.
20. *Caro CG, Fitz-Gerald JM, Schroter RC*. Arterial Wall Shear and Distribution of Early Atheroma in Man. Nature 1969; 223(5211): 1159−61.
21. *Malek AM, Alper SL, Izumo S*. Hemodynamic shear stress and its role in atherosclerosis. JAMA 1999; 282(21): 2035−42.
22. *Friedman MH, Bargeron CB, Deters OJ, Hutchins GM, Mark FF*. Correlation between wall shear and intimal thickness at a coronary artery branch. Atherosclerosis 1987; 68(1−2): 27−33.
23. *Prosi M, Perktold K, Ding Z, Friedman MH*. Influence of curvature dynamics on pulsatile coronary artery flow in a realistic bifurcation model. J Biomech 2004; 37(11): 1767−75.
24. *Chatzizisis YS, Baker AB, Sukhova GK, Koskinas KC, Papafaklis MI, Beigel R*, et al. Augmented expression and activity of extracellular matrix-degrading enzymes in regions of low endothelial shear stress colocalize with coronary atheromata with thin fibrous caps in pigs. Circulation 2011; 123(6): 621−30.
25. *Koskinas KC, Sukhova GK, Baker AB, Papafaklis MI, Chatzizisis YS, Coskun AU,* et al. Thin-capped atheromata with reduced collagen content in pigs develop in coronary arterial regions exposed to persistently low endothelial shear stress. Arterioscler Thromb Vasc Biol 2013; 33(7): 1494−504.
26. *Rizik DG, Klassen KJ, Hermiller JB*. Bifurcation coronary artery disease: current techniques and future directions (part 2). J Invasive Cardiol 2008; 20(3): 135−41.
27. *Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ*, et al. Atherosclerotic Lesion Size and Vulnerability Are Determined by Patterns of Fluid Shear Stress. Circulation 2006; 113(23): 2744−53.
28. *Schaar JA, Muller JE, Falk E, Virmani R, Fuster V, Serruys PW,* et al. Terminology for high-risk and vulnerable coronary artery plaques. Report of a meeting on the vulnerable plaque, June 17 and 18, 2003, Santorini, Greece. Eur Heart J 2004; 25(12): 1077−82.
29. *Nakazawa G, Yazdani SK, Finn AV, Vorpahl M, Kolodgie FD, Virmani R*. Pathological findings at bifurcation lesions: The impact of flow distribution on atherosclerosis and arterial healing after stent implantation. J Am Coll Cardiol 2010; 55(16): 1679−87.
30. *Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, Glagov S*. Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. Circ Res 1983; 53(4): 502−14.
31. *Grottum P, Svindland A, Walloe L*. Localization of atherosclerotic lesions in the bifurcation of the main left coronary artery. Atherosclerosis 1983; 47(1): 55−62.
32. *Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E*, et al. Pathology of drug-eluting stents in humans: Delayed healing and late thrombotic risk. J Am Coll Cardiol 2006; 48(1): 193−202.
33. *Kraemer BF, Schmidt C, Urban B, Bigalke B, Schwanitz L, Koch M*, et al. High shear flow induces migration of adherent human platelets. Platelets 2011; 22(6): 415−21.
34. *Gutierrez-Chico JL, Gijsen F, Regar E, Wentzel J, de Bruyne B, Thuesen L,* et al. Differences in neointimal thickness between the adluminal and the abluminal sides of malapposed and side-branch struts in a polylactide bioresorbable scaffold: evidence

in vivo about the abluminal healing process. JACC Cardiovasc Interv 2012; 5(4): 428−35.

35. *Jimenez JM, Davies PF.* Hemodynamically driven stent strut design. Ann Biomed Eng 2009; 37(8): 1483−94.
36. *Raber L, Juni P, Loffel L, Wandel S, Cook S, Wenaweser P,* et al. Impact of stent overlap on angiographic and long-term clinical outcome in patients undergoing drug-eluting stent implantation. J Am Coll Cardiol 2010; 55(12): 1178−88.

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CURRENT TOPIC

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Pharmacovigilance as an imperative of modern medicine – experience from Montenegro

Farmakovigilanca kao imperativ moderne medicine: iskustvo iz Crne Gore

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pharmacovigilance; drug utilization review; adverse drug reaction reporting systems; drug utilization.

Ključne reči:
farmakovigilanca; lekovi, korišćenje, izveštaji; lekovi, neželjeno dejstvo, sistemi za izveštavanje; lekovi, korišćenje.

Introduction

According to the definition of World Health Organization (WHO), pharmacovigilance is a science that comprises activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problem [1]. The specific aims of pharmacovigilance are to improve patient care and safety in relation to the use of medicines and all medical and paramedical interventions, improve public health and safety in relation to the use of medicines, contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use, and promote understanding, education and clinical training in pharmacovigilance and its effective communication to the public [2].

According to the latest definition, denounced in 2010, adverse effect is harmful and unintentionally caused reaction to a medicine [3]. Previous definitions of adverse effects [4] (since 1972) meant "adverse and unexpected response to a drug, which occurs in the application of the dose conventionally used for the prophylaxis, diagnosis or treatment of diseases or modifying the physiological functions". The latest definition implies harmful and unintended effects caused by a medicine at any dose [3].

Since its founding the WHO has the mandate to establish international standards for medicines. The realization of this task seriously started during the late sixties, after the discovery of the reproductive toxicity of thalidomide. A pilot project to establish an international system of monitoring adverse drug reactions (1968) made very quick recommendations on the establishment of national centers to deal with this issue and stressed the necessity of setting uniform guidelines. The WHO Collaborating Center for International Drug Monitoring was founded in 1978 and located in Uppsala (Sweden) [5], and contact with this center is the most important task of national pharmacovigilance centres. Today this center has 118 regular members and 30 affiliated member states. The most important sources of information on adverse drug reaction (ADR) are spontaneous reporting by healthcare workers, systematic study on the whole population and analysis of health statistics and information on the consumption of medicines. Data from these sources would be poured in a reference center. At this point, Uppsala base has over ten million reported cases of adverse effects of medicines from around the world [6].

The importance of monitoring of the safe use of medicines

The aim of the activities on monitoring and collecting data on ADRs is rationalization of pharmacotherapy, use of the most effective medicine with the least ADR upon the establishment of the proper diagnosis.

ADRs may be observed during preclinical and clinical trials. Data collected during these phases of drug development cannot predict a possible adverse ADR that may manifest only after placing a medicinal product on the market. The reasons are as follows: animal studies are insufficient to predict the safety of medicines in humans; in clinical trials a limited number of selected patients is included, conditions of

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administration of medicine are different from those in normal clinical practice, and duration of trials is limited; less than 5,000 patients would be exposed to medicine during clinical trials before its placing on the market and only ADRs with higher incidence of manifestation could have been observed; at least 30,000 patients need to take medicine to observe ADR with the incidence of 1 : 10,000; data on rare serious adverse events, toxic effects of chronic treatment, the use of a medicine in specific categories of patients (children, the elderly, pregnant women) or interactions with other medicines are often incomplete or not available [7].

It is estimated that in the first three phases of clinical trials of the medicine less than 0.1% of all ADRs would be detected, although there are opinions that the number is higher, up to 2% [8].

The most important source of new information on the unknown effects of medicines before its registration represents the fourth phase of clinical testing or monitoring of medicines. It starts after placing a medicine on the market and indicates that the medicine is in widespread, general use. This phase lasts indefinitely. In this open-ended period, both harmful and beneficial unknown aspects of the drug will be revealed. In the fourth phase, ADRs which rarely occur are registered, for example, thrombocytopenia caused by sulfonamides (only in one in 15,800 patients); thrombocytopenic purpura, which follows the use of clopidogrel, occurs in one in a million of those who use it [9]. This low frequency of purpura is not the reason for restrictions of routine use of clopidogrel, unlike ticlopidine, a medicine of the same group, in which purpura occurs in one of 2,000 to 4,000 patients who receive it. These examples show that the correct conclusion regarding frequency of ADRs need many patients and a very long period of monitoring the effects of the drug.

After placing a medicine on the market, manufacturers are obliged to monitor its safety, but this does not necessarily imply the organization of prospective studies about its ADRs. These data manufacturers receive mostly from physicians who prescribe/apply medicine, pharmacists and other healthcare workers. If they do not participate in these activities, physicians and pharmacists eliminate their personal contribution to the extension of knowledge of ADRs. Reporting ADRs, healthcare workers can protect health of their patients.

Pharmacovigilance is the need and obligation of every country, because there are differences in the incidence of ADRs (and other problems caused by medicines) among different communities. Causes may be as follows: differences in the prevalence of certain diseases; different practice of prescribing medicines; genetic factors, diet, habits; different manufacturing process which affects the quality and composition of medicines; differences in the use of the drug, including the therapeutic indication and the dosage regimen; co-administration of the traditional and herbal products which may cause specific toxicological problems, whether administered alone or in combination with other medicines [10].

Information collected in one country (for example in the country of the manufacturer) may not be relevant to other regions of the world, because the conditions of administration could be different [7].

Monitoring the safety of medicines on the market is a valuable tool for detecting ADRs that are the result of counterfeiting or inadequate quality of a medicine.

An organized and permanent monitoring of the effects of medicines after obtaining marketing authorisation is necessary to recognize and prevent ADRs on time.

In consideration of aforementioned, the greatest importance in obtaining information about ADRs after granting marketing authorization has spontaneous reporting of adverse reactions.

Some of numerous recent examples experiencing contemporary pharmacotherapy with its accompanying risks manifested in the form of ADRs are shown in Table 1.

Decision of withdrawal of these medicines from the market due to the unfavourable ratio of benefits and risks was made by the national regulatory authorities based on data collected by spontaneous reporting of adverse reactions.

Table 1

Examples of medicines withdrawn from the market on the basis of decisions made by regulatory authorities as the result of spontaneous reporting of adverse drug reactions (ADRs) [11–13]

Year	Medicine	Pharmacotherapeutic group	Reason of withrawal
2000	astemizole	histamine H_1-receptor antagonist	QT prolongation
2000	troglitazone	antidiabetic (thiazolidinedione)	hepatotoxicity
2000	cisapride	serotonin 5-HT4 agonist histamine H_2-receptor antagonist	QT prolongation
2001	cerivastatin	statin	rhabdomyolysis
2001	trovafloxacin	fluoroquinolone antibiotic	acute liver failure
2001	rapacuronium	neuromuscular blocker	bronchospasm
2004	rofecoxib	(COX-2) inhibitor	myocardial infarction
2005	hydromorphone	opioid analgesic	the risk of overdose
2005	thioridazine	typical antipsychotic	cardiotoxicity
2006	ximelagatran	anticoagulant	hepatotoxicity
2007	pergolide	dopamine receptor agonist	defect of heart valves
2007	aprotinin	phospholipase A2 inhibitor	cardiac death
2007	insulin inhaled	antidiabetic	unsafe
2009	efalizumab	monoclonal antibody	multifocal leukoencephalopathy
2010	sibutramine	anorexiant	cardiotoxicity
2010	rosiglitazone	antidiabetic (thiazolidinedione)	myocardial infarction
2013	hexoprenaline	β_2-adrenergic receptor agonist	cardiac disorders

COX-2 – cyclooxygenase 2.

The frequency and significance of adverse drug reactions

In the last few decades, numerous studies showed an increase in morbidity and mortality caused by medicines. It is estimated that the adverse effects of medicines are the fourth to sixth leading cause of mortality in the United States [14, 15].

ADRs appear more frequently than actually reported and registered, and the consequences are complex and mostly have a medical, economic and social importance [7].

In some countries, the number of hospitalizations due to ADRs is about 10% [16–18]. In the European Union (EU), the average frequency of ADRs in adults is 1 *per* 30–60 visits to the doctor, or 1 *per* 30–40 patients [19]. In children, the incidence of ADRs varies between 1/60 and 1/83. Data on the frequency of ADRs depend on local legislation, the accepted definition in the field of pharmacovigilance, the national policy of prescribing drugs, the methods used in the detection of ADR, the institution of the origin of information (hospital or outpatient facilities) and other factors. Thus, drugs are significantly more often prescribed in France and Germany than in other EU countries (90% of visits to a doctor in France, followed by prescribing an average of 4.2 prescriptions, while the average for the EU is 0.8) [20].

ADRs that can be avoided or prevented make up a significant portion (28–80%) of ADRs. In Italy, ADRs that could be prevented caused 1.4% of all hospital admissions. Other authors note that 35.5% of hospital admissions caused by ADRs could be prevented [20]. Generally, it is estimated that ADRs could have been prevented in about 50% of cases [21–24].

ADRs are, also, a common cause of morbidity and mortality within the hospital setting. The hospital environment, with its clearly defined patient population, is an ideal setting to identify potential ADR signals [25].

It has been estimated that 10–30% of hospitalized patients experience ADRs [26–30] and 0.3–10% of all hospital admissions are actually the results of ADRs [17, 22, 31]. In hospital environment, 3% of all fatal outcomes are caused by ADRs [28]. ADRs also cause prolongation of the hospitalization period and increase of hospital costs [27].

Varieties in frequency of ADR occurrence during hospitalization among different studies could be explained by different investigation methods. While in some studies only spontaneously reported ADRs were recorded, in others, ADRs were recorded by using intensive monitoring systems [17, 32]. Furthermore, there are significant differences between stimulated *versus* non-stimulated reporting systems, as well as between manual and electronic active monitoring systems [32]. Prospective collection of ADRs has many advantages over retrospective data collection (which rely on chart review) mostly due to most often daily visits by trained healthcare professionals on selected departments, over a restricted time period, in order to obtain records of all patients and suspected events [33–35].

Furthermore, earlier studies emphasised that ADRs could often be prevented if physicians had had possible risk factors in mind [36–38].

Pharmacovigilance legislation in Montenegro

The role of the Agency for Medicines and Medical Devices

Appalling statistics at the level of EU countries, in which the pharmacovigilance system was building through decades, especially when it comes to proven fatalities caused by irrational use of medicines (200,000 deaths annually in the EU due to adverse effects of medicines) [39] and the enormous costs of their treatment (about 709 billion € annually) [39] were the trigger for proposal, final approval by the European Parliament and entry into the force of the new EU regulation on pharmacovigilance.

The Agency for Medicines and Medical Devices of Montenegro (*Crnogorska agencija za lekove i medicinska sredstva* - CALIMS), as a full member of the WHO-Uppsala Monitoring Centre, in order to protect public health by monitoring the safety of medicines, collects, assesses and manages all reported suspected ADRs into the national database, and forwards them to this center.

Reporting of ADR based on the principle of spontaneity means that healthcare workers report any suspected ADR; they should inform the Agency or manufacturer's representative who will forward the report to the Agency. Healthcare workers have moral and professional, but also a legal obligation to do so [40]. Fulfilled reporting form could be submitted to the Agency in one of the following manners: by post, in person, by fax or by e-mail. In 2013 the possibility of reporting through the information system of primary healthcare institutions and general hospitals was introduced. This is expected to be the principal method when it comes to report ADR, because it is an easy, safe and fast way to transfer data from a healthcare institution to the CALIMS.

According to the Law on Medicines, the CALIMS publishes annual report [40] on the results of spontaneous reporting of ADRs. Each new report that arrives at the CALIMS represents important information about medicines and in this sense the CALIMS makes further efforts to work together with other participants in the system of pharmacovigilance in order to build an effective national surveillance system in Montenegro. Special attention is directed towards increasing the number of reports sent by pharmaceutical companies over the person responsible for pharmacovigilance, legally obliged to take an active role in the reporting of ADRs of their medicines placed on the market in Montenegro.

As other agencies for medicines and medical devices, the CALIMS prepares Direct Healthcare Professional Communication (DHPC) [41] – information important for safe and effective use of medicines, which is sent to healthcare professionals by Marketing Authorization Holder (MAH) or the CALIMS. The Agency sends Dear Doctor Letters in case of significant changes in the Summary of product characteristics (new contraindications, lowering recommended dose of medicines, limitations in the indications, limitations in dispensing mode of a medicine, new precautionary measures, etc.), termination of marketing authorization or its temporarily suspension due to safety reasons, or in other similar situations in which it is necessary to inform healthcare

professionals on safe medicine use. Providing information about safe and effective use of medicines is one of the preconditions for their rational use and is considered a public health responsibility. In case Dear Doctor Letter is to be sent by MAH, the content of the letter, as well as plan for communication with healthcare professional must previously be approved by the Agency.

When it appears that a drug leads to frequent and/or unacceptable adverse effects, appropriate regulatory action should be taken: correction of Summary of product characteristics and Patient information leaflet (Level I warning) or withdrawal of the medicine from the market (Level II warning).

Spontaneous reporting and intensive monitoring of ADRs

The Pharmacovigilance Department of the Agency for Medicines and Medical Devices of Montenegro received a total of 106 spontaneous reports of suspected ADR (171 *per* million inhabitants) in 2014, of which 68 reports from healthcare workers, while 38 reports ensued from post-marketing noninterventional studies [42]. The total number of reports increased by 9.28% compared to the year 2013 [43]. Physicians have reported 82% of suspected ADRs, while the pharmacists reported 18% of suspected ADRs. Most reports were received from the Clinical Center of Montenegro (59%) and primary health care system (19%) [42]. The results of spontaneous reporting of ADRs, according to the latest CALIMS annual report [42], indicate that the largest number of reports, according to the the Anatomical Therapeutic Cemical (ATC) classification of suspected drugs, related to drugs belongs to the group of antineoplastic and immunomodulating agents, drugs for cardiovascular system and anti-infectives for systemic use. Reported ADRs [42] based on Medical Dictionary for Regulatory Activities (MedDRA) system organ classification (System Organ Class – SOC) at the most include: skin and subcutaneous tissue disorders (20%), general disorders and administration site conditions (17%), gastrointestinal disorders (11%), laboratory investigations (7%), respiratory, thoracic and mediastinal disorders (6%) and nervous system disorders (5%).

Similar data are listed in the Annual Report on spontaneous reporting of ADRs of Agency for Medicines and Medical Devices of Serbia [44]. According to this report from 2013 the number of reports is also too low, 162.9 *per* million inhabitants [44] (WHO Drug Monitoring Programme defines less than 200 reported ADRs *per* million inhabitants annually as underreporing [45]). Physicians have reported 69% of suspected ADRs, while the pharmacists reported 29%.

In contrast to Montenegro and Serbia, ADR spontaneous reporting in neighboring Croatia is far more common. According to the Annual Report on spontaneous reporting of ADR Agency for Medicinal Products and Medical Devices of Croatia for 2014 [46], a total of 3,112 suspected ADRs was reported, by which Croatia took 16th place out of 115 countries participating in the WHO program of monitoring drug safety [46]. In Croatia, most of the reports came from physicians and pharmacists (the largest number of reports reaches from pharmacies, 35%, followed by the primary health care level facilities and hospitals, 18%).

In order to analyze occurrence, characteristics and risk factors for developing ADRs using intensive monitoring system of ADRs, we conducted a prospective study in 2014, which included 200 patients, hospitalized at Cardiology Center of the Clinical Center of Montenegro [47]. ADRs were collected using a specially designed questionnaire, based on the list of symptoms and signs that could point out to the potential ADR. Data from patients' medical charts, laboratory tests and other available parameters were observed and combined with the data from questionnaire. The results show that 34% of all patients experience at least one ADR. The most common ADRs occurs as nervous system disorders, less frequent are cardiovascular disorders, while immune system disorders are the rarest. Sixteen percent of all ADRs are characterized as serious. The majority of patients (97.3%) recover without consequences. The multivariate analysis shows independent significant associations between ADR and age, gender, co-morbidities, polypragmasia and duration of hospitalization [47].

None of ADRs observed in this study was reported by health workers to the Department of Pharmacovigilance of the Agency, despite legal obligations. Considering a high incidence of ADR in this study and the fact that none observed suspicion of ADR was reported to the CALIMS by health workers, it can be concluded that the system of spontaneous reporting of ADR in Montenegro is deficient.

The CALIMS, as a national representative institution with contacts with European and international databases, remains deprived of valuable information on the safety of medicines that are placed on the market of Montenegro. As each ADR reported by a healthcare worker the CALIMS forwards to the MAH, with the protected data on the health worker who reported the adverse effect, in global document on the safety of drugs, leading pharmaceutical companies have not been included cases from Montenegro.

Futher directions for farmacovigilance development in Montenegro

The success of the pharmacovigilance system of each country depends on the participation of healthcare professionals in it. The CALIMS conducts many activities aimed at the promotion of pharmacovigilance, pointing to the importance of spontaneous reporting of ADR, as well as the training of healthcare professionals in this field. One of them is organizing workshops on pharmacovigilance for the development of a system of continuous monitoring of safety of medicines. The Agency will continue future organizing workshops of this type.

Conclusion

Reporting on ADRs by healthcare workers should be a part of everyday clinical practice, since it is one of the indicators of healthcare quality.

National ADR reporting system in Montenegro is organised by the Pharmacovigilance Department of the Agency for Medicines and Medical Devices of Montenegro, but the number of reports coming from healthcare professionals is quite low.

Therefore, it is necessary to conduct additional training of healthcare workers, to improve their awareness about the importance of ADRs and the risk factors that lead to them, as well as to increase the number of reported suspected ADRs.

R E F E R E N C E S

1. WHO. The importance of pharmacovigilance: Safety monitoring of medicinal products. 2002. Available from: http://apps.who.int/medicinedocs/pdf/s4893e/s4893e.pdf
2. *Düsing R.* Overcoming barriers to effective blood pressure control in patients with hypertension. Curr Med Res Opin 2006; 22(8): 1545−53.
3. Directive 2010/84/EU of the European Parliament and of the Council of 15 December 2010 amending, as regards pharmacovigilance. Available from: http://ec.europa.eu/health/files/eudralex/vol-1/dir_2010_84/dir_2010_84_en.pdf
4. Drug Reactions Database. Available from: http://www.adr- database.com/WhatareADRs.html
5. Uppsala Monitoring Centre. Available from: http://www.who-umc.org/ [cited 2014 September 26].
6. Reporting Trends - Uppsala Monitoring Centre. Available from: http://who-umc.org/DynPage.aspx?id=108476&mn1=7347&mn2=7252&mn3=7322&mn4=7558
7. *Jelenkovic A.* Adverse drug reactions. Belgrade: Hellen J; 2002. (Serbian)
8. *Dukes MN.* Mayler's side effects of drugs. Amsterdam: Elsevier; 1996.
9. *Malgarini RB, Pimpinella G.* Thrombotic thrombocytopenic purpura induced by ticlopidine: The power of "dear health professional" letters. Ann Pharmacother 2000; 34(3): 404−5.
10. Safety of Medicines: A Guide to Detecting and Reporting Adverse Drug Reactions - Why Health Professionals Need to Take Action: Why pharmacovigilance is needed in every country. Available from: http://apps.who.int/medicinedocs/en/d/Jh2992e/5.html
11. *Qureshi ZP, Seoane-Vazquez E, Rodriguez-Monguio R, Stevenson KB, Szeinbach SL.* Market withdrawal of new molecular entities approved in the United States from 1980 to 2009. Pharmacoepidemiol Drug Saf 2011; 20(7): 772−7.
12. European Medicines Agency. Human medicines: Short-acting beta-agonists. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Short-acting_beta-agonists/human_referral_prac_000013.jsp&mid=WC0b01ac05805c516f
13. European Medicines Agency. PRAC - PRAC: Agendas, minutes and highlights. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/document_listing/document_listing_000353.jsp&mid=WC0b01ac05805a21cf
14. *Lazarou J, Pomeranz BH, Corey PN.* Incidence of adverse drug reactions in hospitalized patients: A meta-analysis of prospective studies. JAMA 1998; 279(15): 1200−5.
15. *Chyka PA.* How many deaths occur annually from adverse drug reactions in the United States. Am J Med 2000; 109(2): 122−30.
16. *Levy M, Azaz-Livshits T, Sadan B, Shalit M, Geisslinger G, Brune K.* Computerized surveillance of adverse drug reactions in hospital: Implementation. Eur J Clin Pharmacol 1999; 54(11): 887−92.
17. *Impicciatore P, Choonara I, Clarkson A, Provasi D, Pandolfini C, Bonati M.* Incidence of adverse drug reactions in paediatric in/out-patients: A systematic review and meta-analysis of prospective studies. Br J Clin Pharmacol 2001; 52(1): 77−83.
18. *Nelson KM, Talbert RL.* Drug-related hospital admissions. Pharmacotherapy 1996; 16(4): 701−7.
19. *Todorovic Z, Prostran MJ, Janković S.* Pharmacovigilance In: *Prostran M, Stanulović M, Marisavljević D, Đurić D,* editors. Pharmaceutical medicine. Vršac: Hemofarm AD; 2009. p. 467−516. (Serbian)
20. *Bates DW, Cullen DJ, Laird N, Petersen LA, Small SD, Servi D*, et al. Incidence of adverse drug events and potential adverse drug events. Implications for prevention. ADE Prevention Study Group. JAMA 1995; 274(1): 29−34.
21. *Classen DC, Pestotnik SL, Evans RS, Burke JP.* Computerized surveillance of adverse drug events in hospital patients. JAMA 1991; 266(20): 2847−51.
22. *Peyriere H, Cassan S, Floutard E, Riviere S, Blayac J, Hillaire-Buys D*, et al. Adverse drug events associated with hospital admission. Ann Pharmacother 2003; 37(1): 5−11.
23. *Hallas J, Gram LF, Grodum E, Damsbo N, Brøsen K, Haghfelt T,* et al. Drug related admissions to medical wards: A population based survey. Br J Clin Pharmacol 1992; 33(1): 61−8.
24. *Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ*, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ 2004; 329(7456): 15−9.
25. *Pushkin R, Frassetto L, Tsourounis C, Segal ES, Kim S.* Improving the reporting of adverse drug reactions in the hospital setting. Postgrad Med 2010; 122(6): 154−64.
26. *Dormann H, Muth-Selbach U, Krebs S, Criegee-Rieck M, Tegeder I, Schneider HT*, et al. Incidence and costs of adverse drug reactions during hospitalisation: computerised monitoring versus stimulated spontaneous reporting. Drug Saf 2000; 22(2): 161−8.
27. *Bates DW, Leape LL, Petrycki S.* Incidence and preventability of adverse drug events in hospitalized adults. J Gen Intern Med 1993; 8(6): 289−94.
28. *Smith CC, Bennett PM, Pearce HM, Harrison PI, Reynolds DJ, Aronson JK*, et al. Adverse drug reactions in a hospital general medical unit meriting notification to the Committee on Safety of Medicines. Br J Clin Pharmacol 1996; 42(4): 423−9.
29. *Varenhorst C, James S, Erlinge D, Brandt JT, Braun OO, Man M*, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin-treated patients with coronary artery disease. Eur Heart J 2009; 30(14): 1744−52.
30. *Johnson JA, Bootman JL.* Drug-related morbidity and mortality: A cost-of-illness model. Arch Intern Med 1995; 155(18): 1949−56.
31. *Hallas J, Harvald B, Gram LF, Grodum E, Brøsen K, Haghfelt T*, et al. Drug related hospital admissions: The role of definitions and intensity of data collection, and the possibility of prevention. J Intern Med 1990; 228(2): 83−90.
32. *Thürmann PA.* Methods and systems to detect adverse drug reactions in hospitals. Drug Saf 2001; 24(13): 961−8.
33. *Moore N, Lecointre D, Noblet C, Mabille M.* Frequency and cost of serious adverse drug reactions in a department of general medicine. Br J Clin Pharmacol 1998; 45(3): 301−8.
34. *Martínez-Mir I, García-López M, Palop V, Ferrer JM, Estañ L, Rubio E*, et al. A prospective study of adverse drug reactions as a cause of admission to a paediatric hospital. Br J Clin Pharmacol 1996; 42(3): 319−24.
35. *Francis GS.* Cardiac complications in the intensive care unit. Clin Chest Med 1999; 20(2): 269−85.
36. *Gurwitz JH, Field TS, Harrold LR, Rothschild J, Debellis K, Seger AC,* et al. Incidence and preventability of adverse drug events

among older persons in the ambulatory setting. JAMA 2003; 289(9): 1107−16.
37. *Gurwitz JH, Field TS, Avorn J, Mccormick D, Jain S, Eckler M*, et al. Incidence and preventability of adverse drug events in nursing homes. Am J Med 2000; 109(2): 87−94.
38. *Olivier P, Boulbés O, Tubery M, Lauque D, Montastruc J, Lapeyre-Mestre M*. Assessing the feasibility of using an adverse drug reaction preventability scale in clinical practice: A study in a French emergency department. Drug Saf 2002; 25(14): 1035−44.
39. *European Medicine Agency*. Setting the scene: new European Union Pharmacovigilance legislation. Vienna, Austria: Agencies of the European Union; 2012.
40. Regulation – Low of drugs. "Official Register of Montenegro, no 56/2011". Available from: http://www.sluzbenilist.me/PravniAktDetalji.aspx?tag=%7BEB8D380A-167D-4B1B-BFBB-B6593034D628%7D (Montenegrian)
41. CALIMS. Letters to health care workers. Available from: https://www.calims.me/ (Montenegrian)
42. 2014. Annual Report - servlet1. Available from: http://www.calims.me/Portal/faces/servlet1?_afrWindowMode=0&putanja=Izvje%C5%A1taj+o+prijavljenim+ne%C5%BEeljenim+dejstvima+za+2014.+godinu.pdf&_afrLoop=16645829555640651&_adf.ctrl-state=tgt7xozzi_38 (Montenegrian)
43. 2013. Annual Report - servlet1. Available from: http://www.calims.me/Portal/faces/servlet1?putanja=Izvjestaj+o+prijavljenim+nezeljenim+dejstvima+za+2013_+godinu.pdf&_afrWindowMode=0&_afrLoop=16733522002965960&_adf.ctrl-state=1d37e0oggc_38 (Montenegrian)
44. ALIMS. Godisnji Izvestaj NRL. 2013. Available from: http://www.alims.gov.rs/ciril/files/2014/12/NDL-2013.pdf (Montenegrian)
45. Safety Monitoring of Medicinal Products: Guidelines for Setting Up and Running a Pharmacovigilance Centre: 5. Special Issues in Reporting: 5.3 Under-reporting. Available from: http://apps.who.int/medicinedocs/en/d/Jh2934e/6.3.html
46. 2014 Report on adverse drug reactions. Available from: http://www.halmed.hr/pdf/publikacije/Izvjesce_o_nuspojavama_u_2014.pdf (Croatian)
47. *Mugosa S, Bukumiric Z, Kovacevic A, Boskovic A, Protić D, Todorovic Z*. Adverse drug reactions in hospitalised cardiac patients: characteristics and risk factors. Vojnosanit Pregl 2015; 72(11): 975−81.

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CASE REPORTS

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Pulmonary thromboembolism and sudden death in psychiatric patients – Two cases report

Tromboembolija pluća i iznenadna smrt psihijatrijskih bolesnika

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Abstract

Introduction. Pulmonary thromboembolism occurs usually by running a thrombus from the deep veins of the legs rarely periprostatic or periuteric veins. Virchow's triad of necessary conditions for the occurrence of thrombosis involves disruption of blood flow, disruption of blood chemistry and damage to the vessel wall. Venous thrombosis is often associated with the implementation of antipsychotic therapy. **Case report.** We reported two cases of sudden death of psychiatric patients who were in both cases fixed during hospitalization. The first case was a 26-year-old woman treated a year with the diagnose of postpartum reactive psychosis. She was hospitalized because of mental state worsening with a dominant depressed mood, visual and auditory hallucinations. Her therapy was determined by diazepam, clozapine, haloperidol and lamotrigine. Suddenly, the patient died on the fifth day of hospitalization. The autopsy showed massive thromboembolism of the pulmonary artery branches. Toxicological analysis revealed the presence of therapeutic doses of antipsychotics. The second case was a-45-year-old men, a long-time alcoholic. On admission, the diagnosis of *delirium tremens* was established, and diazepam and haloperidol were administered. On the fifth day of hospitalization, he suddenly died. The autopsy showed thromboembolism of the branch of the pulmonary artery. Toxicological analysis established the presence of nordiazepam in urine (0.06 mg/L). Both patients were fixed during hospitalization. **Conclusion**. Both presented psychiatric patients were younger than 50 years, were not overweight, did not have changes of the venous blood vessels. Nowadays, when the issue of medical responsibility often arises in these and similar cases of sudden death in patients treated in psychiatric clinics, the questions on medical malpractice could be expected.

Key words:
psychotic disorders; antipsychotic agents; immobilization; time factors; venous thrombosis; pulmonary embolism.

Apstrakt

Uvod. Plućna tromboembolija najčešće nastaje pokretanjem tromba iz dubokih vena nogu, ređe iz periprostatičnih ili periuterusnih vena. Virhovljevo trojstvo neophodnih uslova za nastanak tromboze podrazumeva poremećaj protoka krvi, poremećaj hemizma krvi i oštećenje zida krvnog suda. Venska tromboza se često dovodi u vezu sa primenom antipsihotične terapije. **Prikaz bolesnika**. Prikazali smo dve naprasne smrti psihijatrijskih bolesnika koji su bili fiksirani tokom hospitalizacije. Prvi bolesnik bila je žena, stara 26 godina, lečena godinu dana pod dijagnozom postpartalne psihotične reakcije. Hospitalizovana je zbog pogoršanja psihičkog stanja sa dominanatnim depresivnim raspoloženjem, vizuelnim i akustičnim halucinacijama. Određena joj je terapija: diazepam, klozapin, haloperidol i lamotrigin. Naprasno, umrla je petog dana hospitalizacije. Obdukcijom je ustanovljena masivna tromboembolija grana plućne arterije. Toksikološkom analizom ustanovljeno je prisustvo terapijskih doza antipsihotika. Drugi bolesnik bio je muškarac, star 45 godina, dugogodišnji alkoholičar. Na prijemu je bila postavljena dijagnoza *delirium tremens* i ordinirana terapija: diazepam i haloperidol. Petog dana hospitalizacije umro je naprasno. Obdukcijom je ustanovljena tromboembolija grana plućne arterije. Toksikološkom analizom ustanovljeno je prisustvo nordiazepama u urinu (0,06 mg/L). **Zaključak**. U prikazanim slučajevima oba psihijatrijska bolesnika bila su mlađa od 50 godina, nisu bili gojazni, nisu imali promene na venskim krvnim sudovima, a umrli su zbog tromboembolije pluća. U ovom trenutku, kada se pitanje lekarske odgovornosti često postavlja, može se očekivati da se postavi pitanje načina lečenja u ovakvim i sličnim slučajevima iznenadne smrti kod bolesnika lečenih u psihijatrijskim klinikama.

Ključne reči:
psihotički poremećaji; antipsihotici; imobilizacija; vreme, faktor; tromboza, venska; pluća, embolija.

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Introduction

Pulmonary thromboembolism occurs in 95% of cases by initiating thrombus from the deep veins in the legs, or less commonly from periprostatic or periuteric veins. Virchow's triad of necessary conditions for the occurrence of thrombosis involves disorder of blood flow, disruption of blood chemistry and damage to the vessel wall [1]. Clinical practice shows that the thrombosis is often seen in obese, elderly people with disseminated malignant tumors or after surgery. Drugs that influence thrombosis are antipsychotics, usually the first generation, and also contraceptive therapy is mentioned [2]. Based on analysis of autopsy materials, deaths from pulmonary thromboembolism in 31% states the history of psychiatric pathology [3]. Other authors state that pulmonary thromboembolism occurs in psychiatric patients as a cause of sudden death in 4% [4]. Some authors suggest that there may be additional factors along with antipsychotic drugs that cause thrombosis in psychiatric patients, like obesity, stillness, increased level of atiphospholipids, hyperhomocysteinemia, and hyperprolactinemia [4, 5]. We reported two sudden deaths in psychiatric patients, in whom the cause of death, massive pulmonary thromboembolism, was established by forensic autopsy.

Case report 1

A woman, aged 26, with moderately developed musculoskeletal structure, nutritional status mediocre, gave birth to a healthy female child 14 months ago. Her psychological problems began two months following delivery. She was treated for the year as outpatient with antipsychotics (clozapine, lorazepam, lamotrigine, risperidone), diagnosed with *postpartum* reactive psychosis. She had postpartal amenorrhea, but values of hormones or cause of amenorrhea were not tested. Due to the deteriorating mental state and depressed mood dominant, visual and auditory hallucinations, she was hospitalized in a psychiatric institution. The patient was treated by diazepam, clozapine, haloperidol and lamotrigine. Standard blood biochemistry was performed and established elevated sedimentation value of 14 mm/h (reference values: < 20 mm/h), S -glucose 6.4 mmol/L (reference values: 4.1–5.9 mmol/L), and S-cholesterol, 6.55 mmol/L (reference values: < 5.2 mmol/L) and other biochemical parameters were within the reference values. The results of blood tests showed 7.85 × 10^9/L leukocytes [normal range (nr) 3.40–9.70 × 10^9/L] leukocyte counts without deviations, the number of red blood cells also in the reference values of 4.28 × 10/L (nr 3.86–5.08 × 10^{12}/L). Other values, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDWC) were within the reference values, and mean corpuscular volume (MCV), the average volume of red blood cells was slightly elevated 99 fl (nr 83–97 fl). Platelet count was 267 × 10^9/L (nr 158–424 × 10^9/L) and other platelet parameters (plateletcrit – PCT, mean platelet volume – MPW, platelet distribution width – PDWC) were without deviation. Laboratory tests of coagulation factors and D-dimer were not done. Examination by the psychiatrist showed her adequate response to the therapy. On the fifth day of hospitalization, the patient suddenly died. The autopsy showed massive thromboembolism of the pulmonary artery branches (Figure 1). By longitudinal cuts on the back of both legs, blood clots in the veins of both lower legs were observed. (Figure 2). Except cystic ovaries, no pathological changes in other organs were established. Toxicological analysis of blood samples showed the presence of diazepam (0.08 mg/L,) lamotrigin (0.10 mg/L) and temazepam (0,07 mg/L). Toxicological analysis of stomach contents, liver with gall bladder, kidney and brain showed the presence of therapeutic doses of antipsychotics. Mean values were established in these samples for clozapine (0.0526 mg/kg), N-desmethyl clozapine (0.0305 mg/kg), haloperidol (0.0055 mg/kg), lamotrigin (0.098 mg/kg) diazepam (0.0255 mg/kg) and temazepam (0.057 mg/kg).

Case report 2

We presented a 45-year-old man, with moderately developed musculoskeletal structure, nutritional status modera-

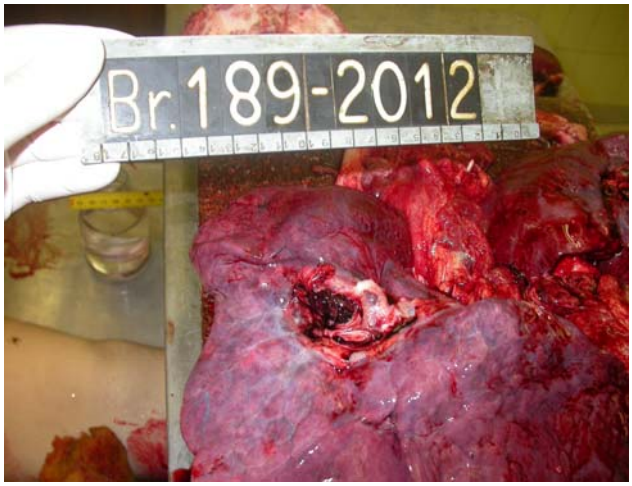

Fig. 1 – Embolus in the pulmonary artery.

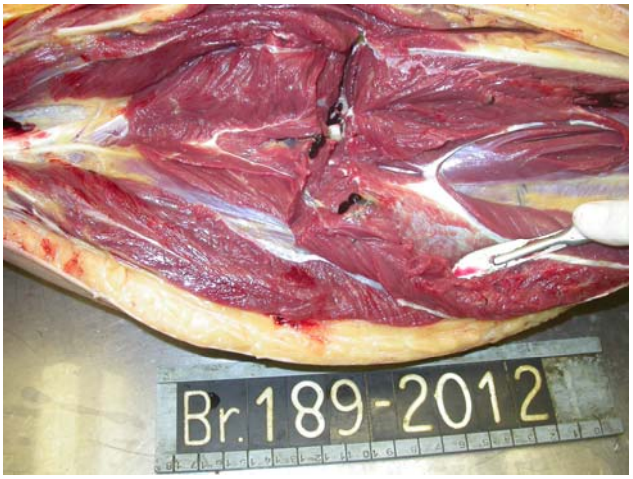

Fig. 2 – Thrombosis of the veins of the lower extremities.

te, long-time alcoholic. The patient was occasionally and irregularly treated as outpatient, receiving diazepam. Due to the deterioration in his mental state, insane ideas, auditory and visual perceptual illusion, the patient was hospitalized in a psychiatric institution. On admission the diagnosis was *delirium tremens*, and administered therapy included diazepam, and haloperidol, and fixation in bed. Laboratory analysis showed elevated values of sedimentation 29 mm/h (reference values < 14 mm/h). Standard biochemical tests showed elevated values of S-AST 93 U/L (reference values < 40 U/L), S–ALT 61 U/L (reference values < 61 U/L) S-cholesterol 8.27 mmol/L (reference values < 5.20 mmol/L) and the other values within the reference range. Blood test showed red blood cells 4.11×10^{12}/L (nr $3.7–5.8 \times 10^{12}$/L), hemoglobin and hematocrit were with no deviations, and the increased value of MCV 100 fl (nr 80–98 fl) and MCH 34.2 pg (nr 26–32 pg). Leukocyte count was 10.9×10^{9}/L (nr $4.1–10.9 \times 10^{9}$/L), in the leukocyte formula granulocytes were increased, 86% (nr 45–70%), whereas the percentage of lymphocytes decreased, 7% (nr 20–40%). The platelet count was 319×10^{9}/L (nr $150–400 \times 10^{9}$/L), and other parameters of platelets (PCT, MPW, PDWC) were without deviations. Laboratory tests of coagulation factors and D–dimer were not performed. On the fifth day of hospitalization the patient suddenly died. The autopsy showed massive thromboembolism of the pulmonary artery branches (Figure 3). The longitudinal incisions along the back of both legs revealed the presence of blood clots in the veins of both lower legs and thighs. Microscopic examination showed fatty alterations of the liver with focal inflammatory infiltration. Other organs showed no pathological changes. Toxicological analysis of urine revealed the presence of nordiazepam (0.06 mg/L).

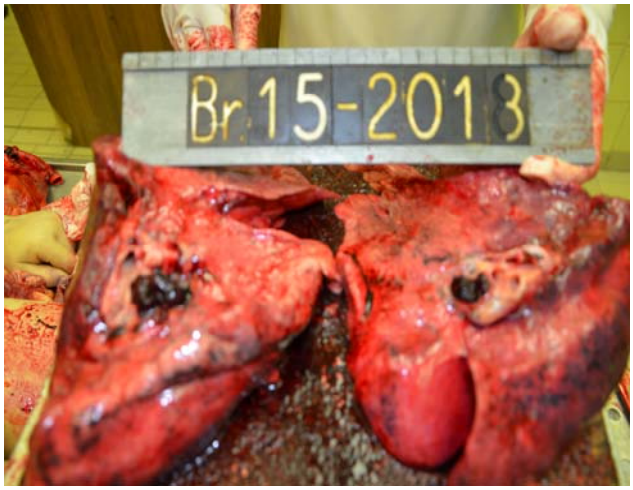

Fig. 3 – Bilateral massive pulmonary thromboembolism.

Discussion

In addition to standard Virchow's triade there are numerous other factors that promote the formation of venous thrombosis. They can be divided into several groups, but the most common is a division of the genetic, acquired diseases and external factors, and combined [4, 5]. Risk factors of venous thromboembolic diseases in psychiatric patients are long-term hospitalization, catatonic conditions, neuroleptic malignant syndrome, limitation of mobility, dehydration, obesity, administration of antipsychotics, hyperprolactinemia, hyperhomocysteinemia, diagnosis of schizophrenia and bipolar affective disorder [5]. The **s**core of risk factor for venous thromboembolism in hospitalized psychiatric patients with reduced mobility gives 2 points for personal history of venous thromboembolism, malignancy, age ≥ 75 years and acute infection, and 1 point to immobilization, physical restraint ≥ 8 h, estrogen therapy, obesity, age 60–74 years, varicose veins, dehydration, thrombophilia and treatment with antipsychotics. In patients with a score of ≤ 3 (low risk) or ≥ 4 (high risk) it is necessary to apply lower extremity exercises, adequate hydration, compressive antithrombotic stocking and in high-risk also heparin therapy [5]. Numerous papers report a connection between treatment with neuroleptics and venous thrombosis, but the exact mechanism of this association is not known. The level of prolactin in plasma was increased in the case shown as thromboembolism in a patient treated with amisulpiride [6]. Antipsychotics are generally dopaminergic antagonists resulting in the increased level of prolactin [7]. The first presented case had amenorrhea for more than a year. The patient was not submitted to hormone testing, nor determining the value of prolactin. Increased risk of venous thrombosis in fixed patients, has already been described, usually after a period of 3–5 days fixation, which corresponds to our cases in who the period of fixation was 5 days [8, 9]. Some authors suggest high doses of antipsychotics and quiescence as a cause of pulmonary thromboembolism in psychiatric patients in the intensive care unit [10]. *In vitro* experiments show that the second generation antipsychotics (olanzapine, clozapine) compared to haloperidol, inhibit platelet aggregation mostly clozapine (21%) and olanzapine (18%) [11]. Statins, postmenopausal hormone replacement, antagonist of vitamin K and oral contraceptives given with antipsychotics, are most often mentioned drugs associated with venous thrombosis [12]. The reported patients did not receive other therapy than antipsychotics. In patients with the diagnosis of schizophrenia on treatment with clozapine and olanzapine, secondary obesity occurs, which can help the development of thrombosis. But obesity, which is not induced by neuroleptics and psychiatric illness is also cited as a predisposing factor for the occurrence of thrombosis [13]. Body mass index (BMI) ≥ 30 kg/m² increases the risk of venous thrombosis 2–5 times [14]. Elevated total serum cholesterol and triglyceride levels were found in patients with thromboembolism as compared to the control group. One explanation of the mechanism is the influence on blood viscosity and erythrocyte aggregation [14]. The presented cases were not obese, but had elevated total cholesterol. As a side effect of antipsychotic drugs, agranulocytosis and leukopenia, were not present in the presented patients [15]. In both presented cases, higher values of MCV and elevated sedimentation value occured. Some authors state that D-dimer and the factor VIII are increased in patients with psychosis without treatment compared to the normal control group [16]. These analyses in our cases were not performed.

Conclusion

Based on the two presented cases of venous thromboembolism in hospitalized psychiatric patients, the potential impact of antipsychotic treatment, given in therapeutic doses, on the occurrence of fatal pulmonary thromboembolism was confirmed, combined with risk factors such as fixing the patient and hypercholesterolemia, even in cases without other well-known risk factors (obesity, older age, changes in veins). In cases with indicated administration of antipsychotic therapy, it is important to reduce the potential impact of other risk factors such as dehydration, elevated cholesterol levels and fixing to the bed, through its reduction to the shortest possible period, exercises of the lower limbs and wearing of elastic bandages. Nowadays, when the issue of medical responsibility often arises, the questions about medical negligence and malpractice can be expected in these and similar cases of sudden death in patients treated in psychiatric clinics.

R E F E R E N C E S

1. *Kuman V, Abbas AB, Fausto N, Mitchel RN.* Robbins Basic Pathology. Ivanović V, editor of the Serbian editions. Belgrade: Data Status; 2010.
2. *Thomassen R, Vandenbroucke JP, Rosendaal FR.* Antipsychotic medication and venous thrombosis. Br J Psychiatry 2001; 179: 63−6.
3. *Lucena J, Rico A, Vasquaez R, Marin R, Martinez C, Salquero M,* et al. Pulmonary embolism and sudden-unexpected death: Prospective study on 2477 forensic autopsies performed at the Institute of Legal Medicine in Seville. J Forensic Leg Med 2009; 14(4): 196−201.
4. *Hägg S, Jönsson AK, Spigset O.* Risk of venous thromboembolism due to antipsychotic drug therapy. Expert Opin Drug Saf 2009; 8(5): 537−47.
5. *Masopust J, Malý R, Vališ M.* Risk of venous thromboembolism during treatment with antipsychotis agents. Psychiatry Clin Neurosci 2012; 66: 541−52.
6. *Skokou M, Gourzis P.* Pulmonary embolism related to amisulpride treatment: a case report. Case Rep Psychiatry 2013; 2013: 718950.
7. *Rang HP, Dale MM, Ritter JM, Moore PK.* Pharmacology. Todorović Z, editor of the Serbian editions. Belgradr: Data Status; 2005.
8. *Dickson BC, Pollanen MS.* Fatal thromboembolic disease: A risk in physically restrained psychiatric patients. J Forensic Leg Med 2009; 16(5): 284−6.
9. *Stefanović V, Kuzmanović A, Stefanović S.* Fatal pulmonary thromboembolism after prolonged physical immobilization in hospitalized psychiatric patients. Vojnosanit Pregl 2013; 70(10): 903−7.
10. *McLnerney SJ, McDonald* C. Idiopathic pulmonary thromboembolism in the course of intensive psychiatric inpatient care: case report and treatment guidelines. BMJ Case Rep 2012; 2012: pii: bcr1220115285.
11. *Dietrich-Muszalska A, Rabe-Jablonska J, Nowak P, Kontek B.* The fisrt and second - generation antipsyhotic drugs affect ADP-induced platelet aggregation. World J Biol Psychiatry 2010; 11(2 Pt 2): 268−75.
12. *Jonsson A, Horvath-Puho E, Hagg S, Pedersen L, Toft SH.* Antipsychotics and risk of venous thromboembolism: A population-base case-control study. Clin Epidemiol 2009; 1: 19−26.
13. *Jönsson AK, Brudin L, Ahlner J, Hedenmalm K, Eriksson A, Hägg S.* Antipsychotics associated with pulmonary embolism in a Swedish medicolegal autopsy series. Int Clin Psychopharmacol 2008; 23(5): 263−8.
14. *Vaya A, Mira Y, Ferrando F, Contreras M, Estelles A, Espana F,* et al. Hyperlipidaemia and venous thromboembolism in patients lacking thrombophilic risk factors. Br J Haemathol 2002; 118(1): 255−9.
15. *Nazer LH, Shankar G, Ali BA, Aj-Najjar T.* Fatal agranulocytosis associated with psychotropic medication use. Am J Health Syst Pharm 2012; 69(10): 863−7.
16. *Masopust J, Maly R, Andryis C, Vališ M, Bažant J, Hosak L.* Markers of thrombogenesis are activated in unmedicated patients with acute psychosis: A matched case control study. BMC Psychiatry 2011; 11: 2.

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CASE REPORT

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Unruptured retroperitoneal pregnancy implanted in the left broad ligament: A case report

Nerupturirana retroperitonealna trudnoća u levom širokom ligamentu

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Abstract

Introduction. Retroperitoneal ectopic pregnancy is extremely rare, but potentially fatal condition due to possible massive hemorrhage, representing a great challenge to clinicians. **Case report.** We presented early retroperitoneal pregnancy in a patient with previous caesarean section, diagnosed at the sixth gestational week, located in the left broad ligament, primary treated by laparoscopy, which had to be converted to laparotomy due to massive intraoperative bleeding from the implantation site. **Conclusion.** High index of suspicion, combined with carefully interpreted clinical and ultrasound findings are crucial for the timely diagnosis of retroperitoneal pregnancy, before the occurrence of severe bleeding. The rising, even plateau of serum β-human chorionic gonadotropin (β-HCG) levels without identification of uterine or ectopic (tubal) pregnancy should cause suspicion on ectopic pregnancy in unusual location.

Key words:
pregnancy, ectopic; retroperitoneal space; laparoscopy; intraoperative complications; gynecologic surgical procedures.

Apstrakt

Uvod. Retroperitonealna ektopična trudnoća je krajnje retko, ali moguće fatalno stanje zbog masivne hemoragije i predstavlja veliki izazov za kliničara. **Prikaz bolesnika.** Prikazali smo ranu retroperitonealnu trudnoću kod pacijentkinje sa carskim rezom u anamnezi, dijagnostikovane u šestoj nedelji gestacije, lokalizovanu u *ligamentum latumu* sa leve strane, primarno lečenu laparoskopski, pri čemu je načinjena konverzija u laparotomiju zbog masivnog intraoperativnog krvarenja sa mesta implantacije. **Zaključak.** Da bi se na vreme postavila dijagnoza retroperitonealne trudnoće, pre pojave obilnog krvarenja, neophodno je da se ima na umu ova mogućnost i pažljivo interpretira klinički i ultrazvučni nalaz. Nivoi β-humanog horionskog gonadotropina (β-HCG) u serumu koji rastu ili održavaju plato, a da nije identifikovana trudnoća u uterusu ili vanmaterična u jajovodu, treba da navedu na pomisao da se radi o ektopičnoj trudnoći neuobičajene lokalizacije.

Ključne reči:
trudnoća, ektopična; retroperitonealni prostor; laparoskopija; intraoperativne komplikacije; hirurgija, ginekološka, procedure.

Introduction

Retroperitoneal pregnancy is a very rare form of ectopic pregnancy. It could be the result of primary retroperitoneal implantation with enigmatic pathogenesis or secondary following tubal rupture in the broad ligament. In modern literature there are less than 25 well-documented cases of primary retroperitoneal pregnancy [1, 2].

We presented a primary retroperitoneal pregnancy implanted in the left broad ligament.

Case report

A 21-year-old *gravida* 1, *para* 1, was admitted into our clinic due to a 6-week history of amenorrhoea, lower abdominal pain and vaginal bleeding. Ectopic gravidity was suspected. Before 18 months the patient had term caesarean delivery performed due to breech presentation, oligoamnion and dystocia followed by an uneventful postoperative course. The women was otherwise healthy, had no history of pelvic inflammatory disease and use of intrauterine devices. Her menarche occurred at 12 and menstrual cycles were regular.

On admission the patient was hemodynamically stable. Examination of her cardiac and respiratory systems was unremarkable. Her abdomen was soft, but with mild inguinal tenderness on the left side. A speculum examination indicated the presence of a single cervix with scarce bleeding from external os and no other pathological findings. Bimanual pelvic examination revealed the slightly enlarged soft uterus

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and a tender palpable mass about 4 cm in diameter, on the left adnexal region. Transvaginal ultrasound examination (Toshiba Nemio XG, 6 MHz) showed the empty uterus with 5 mm endometrial strip (Figure 1). A round cystic mass, 4 × 3 × 2 cm in diameter, filled with heterogeneous content and hypoechogenic structure inside, like gestational sac without fetal pole, 1 cm in diameter, was seen just behind the uterine corpus, on the left side (Figure 2). Color Doppler examination revealed reach vascularisation, with the typical "ring of fire", low resistance blood flow around the described mass (Figure 3). Both ovaries appeared sonographically normal with *corpus luteum* on the left ovary. There was no intraperitoneal fluid in the pouch of Douglas. Her laboratory results were as follows: white blood cells (WBC) 12.8 $\times 10^9$/L, red blood cells (RBC) 4.48 $\times 10^{12}$/L, Hb 123 g/L, hematocrit (Ht) 37.5, platelets (PLT) 368 $\times 10^9$/L. Serum electrolytes, coagulation profile and liver function tests were all within physiological limits. At the day of admission her serum β-human chorionic gonadotropin (β-HCG) level (Abbott test; Architect-Total-β-HCG) was 28.643 mU/mL and the next day quantitative β-HCG level decreased to 27,000 mU/mL. Ectopic gravidity was suspected and diagnostic laparoscopy was performed after the written informed consent was obtained.

Surgery was conducted under general anesthesia, induced by means of propofol as induction agent, fentanyl as an analgesic and rocuronium as a muscle relaxant. Anesthesia was maintained with 1–1.5% end-tidal sevoflurane in 50% : 50% O_2/N_2O mixture at 6 L/min flow. The lungs were ventilated to maintain end-tidal carbon dioxide concentration 30–35 mmHg.

Laparoscopy revealed that the omentum was in slight adhesions with anterior abdominal wall (adhesiolysis was immediately undertaken), the uterus was slightly enlarged and the round retroperitoneal mass, 3 cm in diameter, located in the left broad ligament, behind the left round ligament and the left Fallopian tube was superior (Figure 4). The overlying peritoneum was intact. The pouch of Douglas was empty. Both ovaries and Fallopian tubes macroscopically appeared normal in size and shape and without any pathological fin-

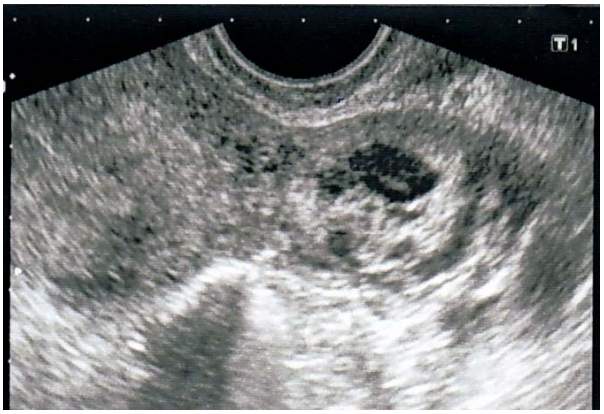

Fig. 1 – Ultrasound image of early retroperitoneal pregnancy in the left broad ligament, just behind the uterine corpus. The uterine cavity is empty.

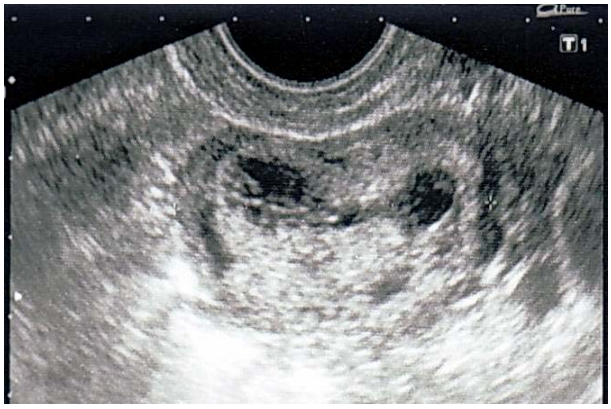

Fig. 2 – Ultrasound image of early retroperitoneal pregnancy in the left broad ligament: a round cystic mass, filled with a heterogeneous content and the hypoechogenic structure inside, like a gestational sac without fetal pole.

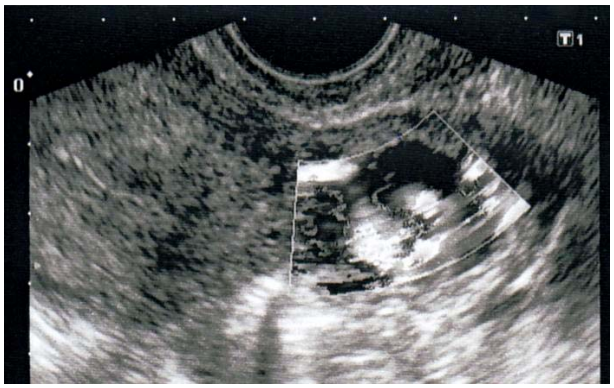

Fig. 3 – Retroperitoneal pregnancy in the left broad ligament: color Doppler examination revealed reach vascularisation with the typical "ring of fire", low resistance blood flow around the described mass.

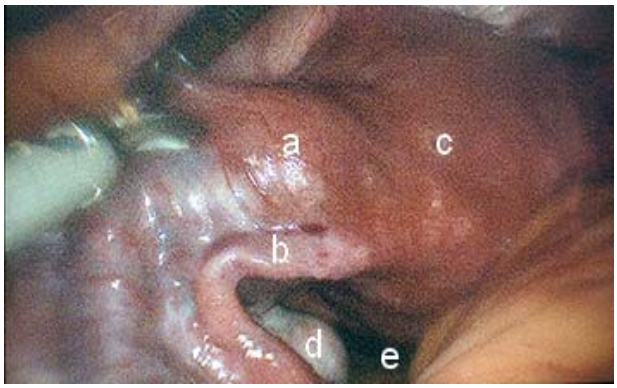

Fig. 4 – Laparoscopic finding of retroperitoneal pregnancy in the left broad ligament at the 6th gestational week: a) round retroperitoneal mass with the intact overlying peritoneum; b) the left Fallopian tube is superiorly, macroscopically normal in size and shape; c) the uterus is slightly enlarged; d) the part of the left ovary, without any pathological findings; e) the pouch of Douglas is empty.

dings. The *corpus luteum* was located on the left ovary. There was no bleeding from the fimbria bilaterally. The peritoneum was opened above the described mass using the ultracision, and evacuation of ovulary tissue was started, but suddenly significant bleeding appeared and urgent laparotomy was immediately performed. There was about 500 mL of fresh blood in the abdomen. The bleeding was controlled by two finger digital compression of the left broad ligament and the remaining ovulary tissue was removed. There were no macroscopic signs of communication or fistula between the described mass and the uterine cavity or the left Fallopian tube. Hemostasis was completed with hemostatic sutures and the abdomen was closed with drainage placed in the pouch of Douglas. Postoperative course was uneventful. Serum β-HCG levels decreased to 750 mU/mL two days after the surgery and became negative after seven days. The histopathology report confirmed ectopic gravidity (Figure 5).

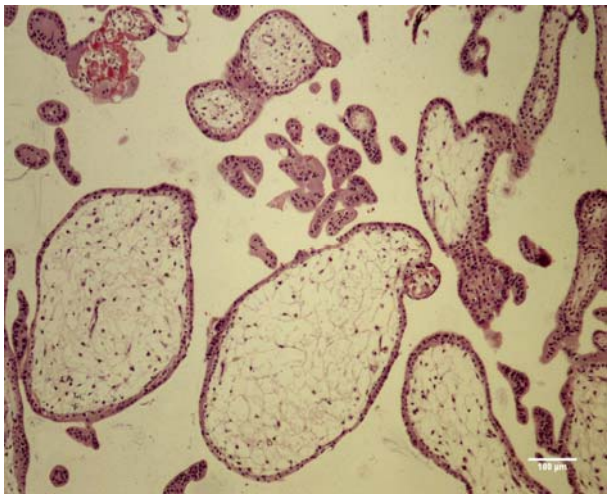

Fig. 5 – Histopathology report confirmed ectopic gravidity: chorionic villi (haematoxylin-eosin, ×100).

Discussion

The incidence of ectopic pregnancy is 0.25–1% of all pregnancies. More than 95% of all ectopic pregnancies are tubal pregnancies. The incidences of extratubal ectopic pregnancies are as follows: abdominal in 1.3%, ovarian and cervical in less than 1% of all ectopic pregnancies [3]. Retroperitoneal ectopic pregnancy, as a subcategory of abdominal pregnancy, is exceptionally rare. The true incidence of retroperitoneal pregnancy is unknown mainly due to false recognizing of abdominal pregnancies with trophoblast invasion as retroperitoneal [1]. The first report of retroperitoneal pregnancy was the case of the broad ligament ectopic pregnancy described almost two hundred years ago by Loschge [4]. Our own review of the literature (Medline data base, through electronic searches without language restriction) showed a total of 65 reported cases of retroperitoneal pregnancies during the last 57 years, with 26 well documented cases during the last 15 years (Table 1). The largest series of the broad ligament retroperitoneal pregnancies (62 cases) was reported by Champion and Tessitore [6] with the incidence of one in 183,900 pregnancies.

The sites of retroperitoneal ectopic implantation include the broad ligament, obturator fossa, areas around large retroperitoneal blood vessels, even the upper retroperitoneal space – attached to the head of pancreas and major blood vessels [7–10]. There have been also reports on broad ligament twin pregnancaes [11, 12] and heterotopic pregnancies involving the broad ligament and the uterus [13] or broad ligament and interstitial pregnancy [14]. Occurrence of partial hydatiform molla was also reported in intraligamentous pregnancy [15].

The most often among retroperitoneal pregnancies is the one located in the broad ligament or intraligamentous pregnancy. The original anatomical relationships for diagnosing broad ligament ectopic pregnancy are: location of the uterus medially, the pelvic side walls laterally, the pelvic floor inferiorly and the Fallopian tube superiorly [6]. Recently, original criterions are fulfilled with the statement that overlying peritoneum should be intact in order to confirm the diagnosis of true retroperitoneal implantation [1]. Our reported case fulfills all the mentioned criteria.

Retroperitoneal ectopic implantation could appear after spontaneous conception [1, 4, 8, 11, 16–26], intrauterine inseminaton [27] or after *in vitro* fertilization/pre-embrio transfer (IVF/ET) [2, 7, 28] (Table 1). Intrauterine device (IUD) *in situ* was found in 8% of abdominal pregnancies, so it is speculated that IUD could be a factor contributing to the development of abdominal pregnancy [29].

Retroperitoneal pregnancy could be the result of primary retroperitoneal implantation or secondary following tubal rupture or trophoblast invasion in the broad ligament. There is also the possibility of primary interstitial and secondary retroperitoneal pregnancy [20].

The pathogenesis of primary retroperitoneal pregnancy is quite obscure. It seems that in the majority of cases, primary retroperitoneal implantation could appear after uterine or tubal surgery that could develop a communication in the retroperitoneal space resulting with the passage of fertilized ovum after spontaneous conception or after IVF/ET (Table 1). The fistulous tract could be developed after termal injury during laparoscopic salpyngectomy [1], after classical salpyngectomy or salpingoophorectomy or due to inappropriate healing of the uterine wall after cesarean section [16, 30]. Spontaneous migration of the embryo from the uterus to the retroperitonel space through these communications could result in retroperitoneal pregnancy.

There is also the possibility of false passage during embrio transfer and placement of embryos into the retroperitoneal space in cases of retroperitoneal pregnancies after IVF/ET [7, 28].

The theory of spontaneous migration of the embryo from the uterus to the retroperitoneal space along lymphatic channels was based on findings of trophoblast surrounded by lymphatic tissue [10, 31]. The contrast-enhanced computed tomography was used to demonstrate the route of embryo migration in retroperitoneal ectopic pregnancy providing further evidence in support of the proposed embryo migration mechanism *via* lymphatic vessels [32].

Table 1

Summarized cases of retroperitoneal pregnancies reported in the last 15 years

Authors	History (previous surgery)	Duration of amenorrhoea / conception	Site of implantation	Treatment
Rama et al. (2015) [16]	caesarean section before 18 months	12 weeks / spontaneous	right broad ligament	laparotomy
Protopapas et al. (2014) [1]	laparoscopic right salpingectomy for EP	6 weeks / spontaneous	right broad ligament	laparoscopy
Salomón et al. (2013) [48]	curretage	15 weeks live / spont.	right broad ligament	laparotomy
Sagili et al. (2013) [17]	unknown	20 weeks / spontaneous	broad ligament	MTX / laparotomy
Rudra et al. (2013) [30]	caesarean section before 4 years	39 weeks non viable fetus	right broad ligament	laparotomy
Singh et al. (2012) [41]	bilateral tubal laparo-ligation	36 weeks live birth	right broad ligament	planned cs
Yoon et al. (2012) [18]	no surgery	5 weeks 2 days / spont.	between the IVC and ureter.	laparotomy
Martínez-Varea et al. (2011) [27]	no surgery	6 weeks / after IUI	next to left uterosacral lig.	laparoscopy
Seckin et al. (2011) [19]	unknown	39 weeks live birth / spont.	broad ligament	planned cs
Persson et al. (2010) [10]	-	-	right obturator fossa	laparoscopy
Milićević et al. (2010) [20]	no surgery	* / spontaneous	**	laparotomy
Okorie et al. (2010) [21]	no surgery	6 weeks 5 days / spont.	close to the blood vessels	laparotomy
Abdul et al. (2008) [22]	right salpingo-ophorectomy	22 weeks / spontaneous	left iliac fossa	laparotomy
Abdul et al. (2008) [22]	no surgery	6 months / spontaneous	right iliac fossa	laparotomy
Bae et al. (2009) [49]	laparoscopic right cornual resection	7 weeks 5 days	anterior aspect of IVC	laparoscopy
Lin et al. (2008) [8]	appendectomy	7 weeks / spontaneous	right obturator foramen area	laparotomy
Chang et al. (2008) [23]	no surgery	spontaneous	left paracolic sulcus	laparoscopy
Holzhacker et al. (2008) [24]	no surgery	18 weeks / spontaneous	right intraligamentary	laparotomy
Apantaku et al. (2006) [28]	bilateral salpyngectomy	following IVF	broad ligament	laparotomy
Cormio et al. (2006) [25]	left salpyngooophorectomy	spontaneous	left broad ligament	laparotomy
Lee et al. (2005) [26]	no surgery	6 weeks / spontaneous	left para-aortic region	laparotomy
Siow et al. (2004) [5]	unknown	10 weeks / spontaneous	right broad ligament	laparoscopy
Reid and Steel (2003) [2]	bilateral salpingectomy for EP	53 day / after IVF	bifurcation of the common IA	laparotomy
Phupong et al. (2003) [50]	two caesarean section	11 weeks	right broad ligament	laparotomy
Dmowski et al. (2002) [7]	bilateral salpyngectomy	41 day after IVF/ET	the head of pancreas	laparotomy
Phupong et al. (2001) [11]	right salpyngectomy	11 weeks / spontaneous	left broad ligament, twin	laparotomy

Abbreviations: * discovered in the 18th week, laparotomy in the 39th week, viable fetus; ** primary interstitial and secondary intraligamentous; EP – ectopic pregnancy; MTX – methotrexate; IA – iliac artery; IVC – inferior vena cava; IVF – *in vitro* fertilization; ET – embrio transfer; IUI – intrauterine insemination; spont. – spontaneous; planned cs – planned caesarean section.

In the case we reported here, lymphatic tissue was not found around the trophobalast, even after careful histopathological examination, so we speculate that the development of the described left broad intraligamentous pregnancy could be explained by spontaneous migration of the embryo from the uterus to the retroperitoneal space through the microscopic fistulous tract caused by inappropriate healing of the uterine wall after the previous caesarean section. Still, we could not exclude with the certainty the possibility of embryo migration *via* lymphatic vessels, taking into account the localization of described retroperitoneal pregnancy.

The preoperative diagnosis of retroperitoneal pregnancy represents the challenge for clinicians. In fact, in the most cases, the diagnosis is made during surgery.

Maternal morbidity and mortality associated with abdominal, especially retroperitoneal, pregnancies could be reduced by early diagnosis. Transvaginal ultrasound examination is the main tool in the diagnostic of an early abdominal (and retroperitoneal) pregnancy. The proposed criteria are: the absence of an intrauterine gestational sac; the absence of tubal dilatation or complex adnexal mass; a gestational sac surrounded by loops of the bowel and separated from the uterus; and a wide mobility of the gestational sac [33, 34]. In fact, sonographic appearance of an early retroperitoneal pregnancy depends on its location. Usually it is fixed deep within the pelvis and not mobile as pregnancy in the non-communicating horn of the unicornuate uterus (cornual pregnancy) [35]. The absence of communication between gestational sac and endometrial cavity differentiates the retroperitoneal broad ligament pregnancy from the pregnancy in non-communicating horn of the unicornuate uterus (cornual pregnancy) and interstitial ectopic pregnancy 35, which was also the truth in the case we reported here. The absence of myometrial layer around this retroperitoneal broad ligament pregnancy differentiates it from interstitial pregnancy. If early retroperitoneal pregnancy is located outside the pelvis, transvaginal ultrasound examination is helpless, and other diagnostic tools, as magnetic resonance imaging (MRI) and other imaging techniques, must be applied.

The suspicion is crucial for the timely diagnosis of retroperitoneal ectopic pregnancy. Rising β-HCG levels, or plateau, without identification of uterine or ectopic (tubal) pregnancy should cause suspicion on ectopic pregnancy in unusual location. In case we reported here, the diagnosis of ectopic pregnancy was made when the patient was still hemodinamically stable, so we opted for laparoscopic treatment during which the definitive diagnosis of left broad ligament pregnancy was confirmed.

The treatment of retroperitoneal pregnancy also represents a great challenge for clinicians. The most of retroperitoneal pregnancies are diagnosed and removed during the early stages of gravidity, but there are reports on broad ligament pregnancies with viable term fetuses [19, 20, 30, 36–41], even *post* term [42].

The great majority of such cases are discovered on surgery for caesarean section.

In spite of many reports on abdominal pregnancies with viable fetuses advanced to term, the risk for the mother is still very high, especially in cases of retroperitoneal pregnancies with the close proximity to large vessels. Immediate surgery is indicated for abdominal pregnancies prior to 23 to 24 weeks because of the high incidence of maternal morbidity and a poor prognosis for the fetus [43].

Fetal anomalies or deformities (facial and cranial asymmetry, joint deformities, CNS anomalies) are associated problem in such pregnancies [41]. However, there is a reported case of successful secondary retroperitoneal pregnancy in which the diagnosis had been suspected during the 18th week, discarded due to lack of symptoms and advanced to term with normal course [20].

The main concern with retroperitoneal pregnancy is associated with possible fatal bleeding due to the proximity of large blood vessels. This is also the possibility during the surgery after the attempt to remove the ectopic pregnancy. In the most of the reported cases laparotomy was the treatment. Nowadays, it seems that most unruptured early nontubal pregnancies could be managed laparoscopically. Laparoscopic treatment of ectopic pregnancy is minimally invasive procedure associated with lower cost, shorter hospital stay and faster recovery. However, the minority of reported cases of retroperitoneal pregnancies are treated laparoscopically. Laparoscopy is suitable for hemodynamically stable patients. Laparoscopic surgery has limitations as unnatural hand-eye coordination and impossibility to palpate the organs, especially retroperitoneal, but with improved skills that should not be a problem.

Hemorrhagy during surgery is the most serious complication. Laparoscopically, it could be controlled by instillation of vasopressin [1] or with bipolar electrodes, monopolar scissors and laparoscopic bowel grasper applied across the corneal edge of the uterus [5], or with temporary occlusion of the right hypogastric artery by removable vessel clips to diminish the risk of bleeding complications [10]. There is also the possibility to apply stitches and close the implantation site inside the broad ligament to achieve hemostasis [44]. In spite of that, there is still the risk of massive intraoperative hemorrhage, which was also happened in the case of our patient, so laparoscopy had to be converted to laparotomy. It seems logical that the extent of intraoperative bleeding depends on the viability and vascularisation of retroperitoneal pregnancy. In the case of our patient, color Doppler ultrasound examination revealed reach vascularisation and the level of serum β-HCG before the surgery was high for ectopic pregnancy at 6th gestational week (over 20 000 mU/mL), both suggesting the vitality of pregnancy which could explain massive intraoperative hemorrhage. Histopathological examination excluded gestational trophoblast disease in the reported case, already extremely rare in ectopic pregnancy [45], with the prevalence of 0.16 : 1000 deliveries [46].

Preoperative use of methotrexate in cases of nonruptured retroperitoneal ectopic pregnancies could diminish intraoperative blood loss [47]. Medical treatment of retroperitoneal pregnancy with methotrexate was reported with variable success [17, 21]. In cases of ectopic tissue adherent around the major vessels, not removed *in toto*, methotrexate treatment is possible [2].

Conclusion

Retroperitoneal ectopic pregnancy is rare, but potentially fatal condition due to possible massive hemorrhage, representing a great challenge to clinicians. The early diagnosis and appropriate surgery are *conditio sine qua non* for succesfull treatment.

High index of suspicion, combined with carefully interpreted clinical and ultrasound findings are crucial for the timely diagnosis, before the occurrence of severe bleeding.

The rising even plateau of β-HCG levels without identification of uterine or ectopic (tubal) pregnancy should cause suspicion on ectopic pregnancy in unusual location.

REFERENCES

1. *Protopapas A, Akrivos N, Athanasiou S, Chatzipapas I, Domal A, Loutradis D.* Ultrasound-assisted intraoperative localization and laparoscopic management of a previously missed unruptured retroperitoneal ectopic pregnancy. Gynecol Surg 2014; 11(3): 207−11.
2. *Reid F, Steel M.* An exceptionally rare ectopic pregnancy. BJOG 2003; 110(2): 222−3.
3. *Bouyer J.* Sites of ectopic pregnancy: A 10 year population-based study of 1800 cases. Hum Reprod 2002; 17(12): 3224−30.
4. *Loschge.* Arch F Med Erfahr 1818; 2: 218.
5. *Siow A, Chern B, Soong Y.* Successful laparoscopic treatment of an abdominal pregnancy in the broad ligament. Singapore Med J 2004; 45(2): 88−9.
6. *Champion PK, Tessitore NJ.* Intraligamentary pregnancy: a survey of all published cases of over 7 calendar months, with the discussion of an additional case. Am J Obstet Gynecol 1938; 36(5): 281−93.
7. *Dmowski WP, Rana N, Ding J, Wu WT.* Retroperitoneal subpancreatic ectopic pregnancy following in vitrofertilization in a patient with previous bilateral salpingectomy: How did it get there. J Assist Reprod Genet 2002; 19: 90−3.
8. *Lin J, Liu Q, Ju Y, Guan Q, Wu Y, Zheng N.* Primary obturator foramen pregnancy: a case report and review of literature. Chin Med J 2008; 121(14): 1328−30.
9. *You JY, Lee YR, Oak SA, Park JH, Lee EH.* A case of retroperitoneal ectopic pregnancy of obturator fossa. Korean J Obstet Gynecol 2011; 54(12): 830−35.
10. *Persson J, Reynisson P, Måsbäck A, Epstein E, Saldeen P.* Histopathology indicates lymphatic spread of a pelvic retroperitoneal ectopic pregnancy removed by robot-assisted laparoscopy with temporary occlusion of the blood supply. Acta Obstet Gynecol Scand 2010; 89(6): 835−9.
11. *Phupong V, Tekasakal P, Kankaew K.* Broad ligament twin pregnancy: A case Report. J Reprod Med 2001; 46: 144−6.
12. *Deshpande N.* Case Report: Broad ligament twin pregnancy following in-vitro fertilization. Hum Reprod 1999; 14(3): 852−4.
13. *Atalla RK, Murphy PC, Balachandar C.* Combined intrauterine and broad ligament ectopic pregnancy. J Obstet Gynaecol 1997; 17(2): 203.
14. *Olsen ME.* Bilateral twin ectopic gestation with intraligamentous and interstitial components. A case report. J Reprod Med 1994; 39(2): 118−20.
15. *Cordero DR, Adra A, Yasin S, O'Sullivan MJ.* Intraligamentary Pregnancy. Obstet Gynecol Surv 1994; 49(3): 206−9.
16. *Rama C, Lepakshi G, Raju SN.* Broad ligament ectopic pregnancy. J Clin Sci Res 2015; 4: 45−8.
17. *Sagili H, Rani R.* Preoperative Methotrexate in Broad-Ligament Pregnancy: Is There a Role. J Gynecol Surg 2013; 29(2): 102−4.
18. *Yoon SH, Lee JY, Yoo MW, Park SW, Sohn IS, Bae JM.* A case of retroperitoneal ectopic pregnancy. Korean J Obstet Gynecol 2012; 55(1): 59−63.
19. *Seckin B, Turkcapar FA, Tarhan I, Yalcin HR.* Advanced intraligamentary pregnancy resulting in a live birth. J Obstet Gynaecol 2011; 31(3): 260−1.
20. *Milićević S, Jovanović D, Vilendecić Z, Ljubić A, Bozanović T, Niketić L.* Full term interstitial retroperitoneal pregnancy with delivery of a healthy infant. J Obstet Gynaecol Res 2010; 36(4): 869−71.
21. *Okorie CO.* Retroperitoneal ectopic pregnancy: is there any place for non-surgical treatment with methotrexate. J Obstet Gynaecol Res 2010; 36(5): 1133−6.
22. *Abdul M, Tabari A, Kabiru D, Hamidu N.* Broad ligament pregnancy: A report of two cases. Ann Afr Med 2008; 7(2): 86.
23. *Chang Y, Ko P, Yen C.* Retroperitoneal abdominal pregnancy at left paracolic sulcus. J Minim Invasive Gynecol 2008; 15(6): 660−1.
24. *Holzbacker S, Elito JJ, Santana RM, Hisaba W.* Advanced intraligamentary abdominal pregnancy: Case report. Rev Assoc Med Bras 2008; 54(5): 387−9. (Portuguese)
25. *Cormio G, Ceci O, Loverro G, Bettocchi S.* Spontaneous left broad ligament pregnancy after ipsilateral salpingo-oophorectomy. J Minim Invasive Gynecol 2006; 13(2): 84−5.
26. *Lee JW, Sohn KM, Jung HS.* Retroperitoneal ectopic pregnancy. AJR Am J Roentgenol 2005; 184(5): 1600−1.
27. *Martínez-Varea A, Hidalgo-Mora J, Payá V, Morcillo I, Martín E, Pellicer A.* Retroperitoneal ectopic pregnancy after intrauterine insemination. Fertil Steril 2011; 95(7): 2433.e1−3.
28. *Apantaku O, Rana P, Inglis T.* Broad ligament ectopic pregnancy following in-vitro fertilisation in a patient with previous bilateral salpingectomy. J Obstet Gynaecol 2006; 26(5): 474.
29. *Poole A, Haas D, Magann EF.* Early abdominal ectopic pregnancies: a systematic review of the literature. Gynecol Obstet Invest 2012; 74(4): 249−60.
30. *Rudra S, Gupta S, Taneja BK, Gupta R.* Full term broad ligament pregnancy through a Cesarean scar. Obstet Gynecol Sci 2013; 56(6): 404−7.
31. *Hall JS, Harris M, Levy RC, Walrond ER.* Retroperitoneal ectopic pregnancy. J Obstet Gynaecol Br Commonw 1973; 80(1): 92−4
32. *Liang C, Li X, Zhao B, Du Y, Xu S.* Demonstration of the route of embryo migration in retroperitoneal ectopic pregnancy using contrast-enhanced computed tomography. J Obstet Gynaecol Res 2014; 40(3): 849−52.
33. *Gerli S, Rossetti D, Baiocchi G, Clerici G, Unfer V, Di Renzo GC.* Early ultrasonographic diagnosis and laparoscopic treatment of abdominal pregnancy. Eur J Obstet Gynecol Reprod Biol 2004; 113(1): 103−5.
34. *Jurkovic D, Mavrelos D.* Catch me if you scan: ultrasound diagnosis of ectopic pregnancy. Ultrasound Obstet Gynecol 2007; 30: 1−7.
35. *Mavrelos D, Sawyer E, Helmy S, Holland TK, Ben-Nagi J, Jurkovic D.* Ultrasound diagnosis of ectopic pregnancy in the non-communicating horn of a unicornuate uterus (cornual pregnancy). Ultrasound Obstet Gynecol 2007; 30(5): 765−70.
36. *Deneke F.* Advanced abdominal pregnancy in an Ethiopian mother. East Afr Med J 1997; 74(8): 535−6.

37. *Vierhout ME, Wallenburg HC*. Intraligamentary pregnancy resulting in a live infant. Am J Obstet Gynecol 1985; 152(7 Pt 1): 878−9.
38. *Engelhardt W, Elser H*. Ultrasound diagnoses and cardiotocography in case of full-time carried intraligamentous gravidity (author's transl). Geburtshilfe Frauenheilkd 1976; 36(11): 965−9. (German)
39. *Pollmann H*. A full-term, retroperitoneally developed extrauterine pregnancy. Zentralbl Gynakol 1961; 83: 1545−52. (German)
40. *Mittal S, Chhabra P, Khanna R*. Advanced abdominal intraligamentary pregnancy with live birth. Int J Gynaecol Obstet 1994; 46(3): 327−8.
41. *Singh U, Singh N, Sankhwar P*. Full term viable broad ligament pregnancy surgically managed with favorable feto-maternal outcome. J Obstet Gynaecol India 2012; 62(1): 23−4.
42. *Rakotomahenina H, Andrianampy HA, Ramamonjinirina P, Solofomalala GD, Brun JL*. Post-term pregnancy in the broad ligament. Gynecol Obstet Fertil 2014; 42(7−8): 537−9. (French)
43. *Paternoster DM, Santarossa C*. Primary abdominal pregnancy. A case report. Minerva Ginecol 1999; 51(6): 251−3.
44. *Cheung CS, Cheung VY*. Broad ligament ectopic pregnancy. CRSLS e2014.00102.
45. *Hassadia A, Kew FM, Tidy JA, Wells M, Hancock BW*. Ectopic gestational trophoblastic disease: A case series review. J Reprod Med 2012; 57(7−8): 297−300.
46. *Cortés-Charry R, Figueira LM, García-Barriola V, Gomez C, Garcia I, Santiago C*. Gestational trophoblastic disease in ectopic pregnancy: A case series. J Reprod Med 2006; 51(10): 760−3.
47. *Jiang W, Lv S, Sun L, Singer G, Xu C, Lu X*. Diagnosis and treatment of retroperitoneal ectopic pregnancy: review of the literature. Gynecol Obstet Invest 2014; 77(4): 205−10.
48. *Salomón AJ, Ortiz HA, Medrano GM, Rivera ES*. Ectopic intraligamentary pregnancy. Ginecol Obstet Mex M 2013; 81(4): 211−4. (Spanish)
49. *Bae SU, Kim CN, Kim KH, Hwang IT, Choi YJ, Lee M*K, et al. Laparoscopic treatment of early retroperitoneal abdominal pregnancy implanted on inferior vena cava. Surg Laparosc Endosc Percutan Tech 2009; 19: e156−8.
50. *Phupong V, Lertkhachonsuk R, Triratanachat S, Suehlinvong T*. Pregnancy in the broad ligament. Arch Gynecol Obstet 2003; 268(3): 233−5.

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CASE REPORT

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Fahr's syndrome and idiopathic hypoparathyroidism – A case report

Farov sindrom i idiopatski hipoparatireoidizam

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Abstract

Introduction. Fahr´s syndrome is a rare, slowly progressive, neurodegenerative disorder, characterised by extensive, bilateral, and symmetrical basal ganglia calcification. It is associated with neuropsychiatric manifestations and gradually progressive cognitive impairment. Fahr's syndrome is the secondary form of brain calcification that is caused by various metabolic, infectious, or degenerative diseases. **Case report.** We presented a middle-aged male with Fahr's syndrome due to primary idiopathic hypoparathyroidism. Clinical diagnosis was based on signs and symptoms of hypocalcemia, progressive neuropsychiatric illnesses, laboratory evidence of hypoparathyroidism, and radiological signs of calcifications in the basal ganglia. The patient improved after only a few days of intravenous rehydration and calcium substitution, followed by oral supplemental calcitriol. **Conclusion.** Timely recognition of idiopathic and iatrogenic hypoparathyroidism allows appropriate treatment that can prevent the development and clinical manifestations of Fahr´s syndrome and potentially slow its progression.

Key words:
neurodegenerative diseases; calcinosis; basal ganglia; hypoparathyroidism; comorbidity; diagnosis, differential; drug therapy.

Apstrakt

Uvod. Farov sindrom je retko, sporonapredujuće, neurodegenerativno oboljenje sa tipično obostranim i simetričnim kalcifikacijama bazalnih ganglija, koje se najčešće manifestuje neuropsihijatrijskim tegobama sa progresivnim oštećenjem kognitivnih funkcija. Farov sindrom je sekundarni oblik kalcifikacija mozga uzrokovanih raznim metaboličkim, infektivnim ili degenerativnim oboljenjem. **Prikaz bolesnika**. Prikazan je sredovečni bolesnik sa Farovim sindromom uzrokovanim idiopatskim hipoparatireoidizmom. Dijagnoza je postavljena na osnovu progresivnih neuropsihijatrijskih tegoba, laboratorijskih pokazatelja hipokalcemije i hipoparatireoidizma, kao i radiološkog nalaza simetričnih, bilateralnih kalcifikacija, pre svega bazalnih ganglija. Stanje bolesnika bitno je popravljeno i stabilizovano nakon samo nekoliko dana parenteralne-intravenske nadoknade kalcijuma, rehidratacije i primene oralnih preparata kalcitriola. **Zaključak.** Blagovremeno prepoznavanje i lečenje idiopatskog ili jatrogenog hipoparatireoidizma ostavlja mogućnost sprečavanja nastanka, kliničke manifestacije ili bar progresije Farovog sindroma, tj. kalcifikacija bazalnih ganglija.

Ključne reči:
neurodegenerativne bolesti; kalcinoza; bazalne ganglije; hipoparatireoidizam; komorbiditet; dijagnoza, diferencijalna; lečenje lekovima.

Introduction

Fahr's syndrome (FS) is a rare, chronic, slowly progressive, neurodegenerative disorder characterised by extensive bilateral, and symmetrical deposition of calcium in the basal ganglia, thalamus, cerebral cortex, dentate nucleus, cerebellum subcortical white matter, and hippocampus [1–3]. Clinical manifestations occur typically in the fourth or fifth decade of age and usually includes neuropsychiatric manifestations with gradually progressive cognitive impairment [2, 4]. The onset of the disease is usually insidious and frequently is misdiagnosed as a dementia or psychiatric illness.

Hypoparathyroidism (HP) is an endocrine disorder, caused by a heterogeneous group of conditions, in which low calcium and high phosphate levels occur as the result of insufficient parathyroid hormone (PTH) secretion. Idiopathic hypoparathyroidism is a term for a rare deficient PTH secretion without definitive cause and may be genetically inherited or may have an autoimmune cause. Radiologically, this state may cause calcifications, predominantly in *globus pal-*

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lidus of the basal ganglia. Symptoms attributable to their involvement are uncommon at the clinical presentation [5–7].

Histological findings in the form of symmetrical brain calcifications, were observed for the first time by Bomberger in 1855 [8]. Clinical manifestations of Fahr's syndrome was first described in 1930 by German neurologist Karl Theodor Fahr [1, 3, 4, 9]. The association of basal ganglia calcifications with chronic HP, was described for the first time by Eaton et al. [10] in 1939.

Today, there are two entities associated with basal ganglia calcification. The primary form, also called Fahr's disease, is characterised by idiopathic calcifications of brain tissue and it is considered as familial or sporadic disorder. Fahr's syndrome is the secondary form of brain calcifications, that is caused by some other known disease [3, 4, 9, 11, 12].

We presented a middle-aged male with Fahr's syndrome caused by the primary idiopathic hypoparathyroidism.

Case report

A 58-year-old male admitted to our clinic for the history of polymorphic complaints in the form of general weakness, headache, middle back pain, retrosternal chest, and diffuse abdominal pain over the 2-month period. He also had an intermittent episodes of generalised muscle cramps, numbness, tingling, fever, and diarrhea, followed with a mark reduction in body weight. The members of his family noticed some changes in overall behaviour like periodical irritability or disorientation. All of the symptoms occured suddenly two months ago, after upper respiratory tract infection, and were getting worse in the last few days. Physical examination revealed ataxia, bradypsychic response, adynamia, asthenic constitution, edentulousness, slurred speech, mental confusion, and positive Trousseau sign of latent tetany. He had body temperature of 37.5°C, pale skin with no edema or skin lesion. His blood pressure was 100/60 mmHg, with puls rate of 100 beats *per* minute with no organomegaly or lymphadenopathy. The rest of physical examination was unremarkable.

Electrocardiogram revealed episodes of sinus tachycardia with first degree atrioventricular block and slow and prolonged depolarisation (PR interval 214, QT interval over 466 ms) (Figure 1).

Laboratory blood tests showed elevated inflammatory biomarkers: erythrocyte sedimentation rate of 63 mm *per* hour and C-reactive protein level of 36 mg/L (normal range 0.00−3.00 mg/L), normocytic anaemia with haemoglobin value of 109 g/L (normal value 130−180 g/L) and positive urine tests for urinary infection. Biochemistry results included normal concentrations of serum albumin, glucose, bilirubin, cholesterol, potassium, sodium, and serum liver enzyme levels. Serum calcium levels were decreased up to 0.97 mmol/L (normal range 2.13−2.63 mmol/L), ionized calcium level of 0.70 mmol/L (normal range 1.00−1.30 mmol/L) and parathyroid hormone level of 0.44 pmol/L (normal range 1.59−6.89 pmol/L) were also decreased. Serum phosphorus level of 2.00 mmol/L was higher than normal range (0.78−1.65 mmol/L). The results of all other hormone tests were normal.

Multislice computer tomography (CT) of the brain revealed multiple symmetrical large areas of calcification in the basal ganglia, periventricular, supraventricular white matter, as well as in the central regia of pons (Figure 2).

With the exception of hypocalcemia and HP, other secondary causes of brain calcifications were excluded by laboratory testing. Chest radiography and ultrasound examination of the neck, abdomen and pelvis were normal. Osteodensitometry revealed normal bone density. Endoscopic examination of the upper intestine was normal. The ophthalmologist did not see signs of papilledema.

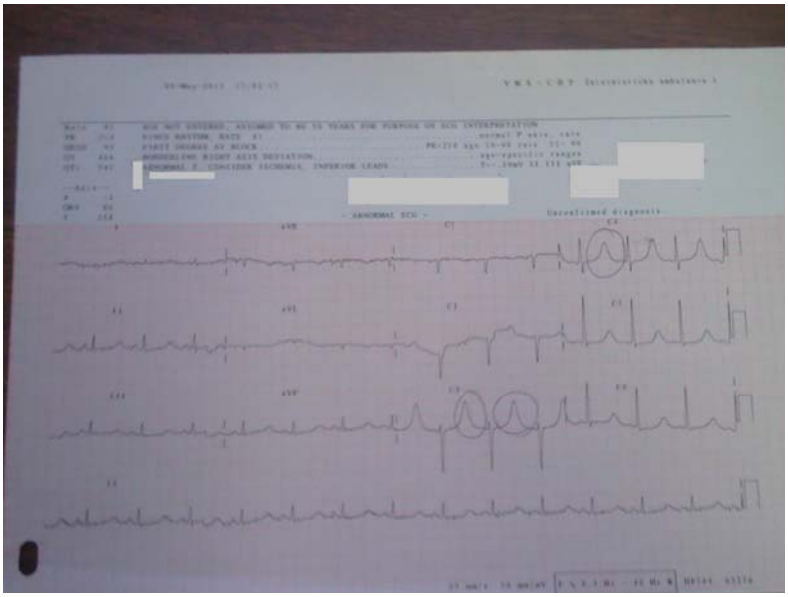

Fig. 1 − Electrocardiogram of a patient with Fahr's syndrome showing first degree atrioventricular block and slow and prolonged depolarisation (PR interval 214 ms, QT interval over 466 ms).

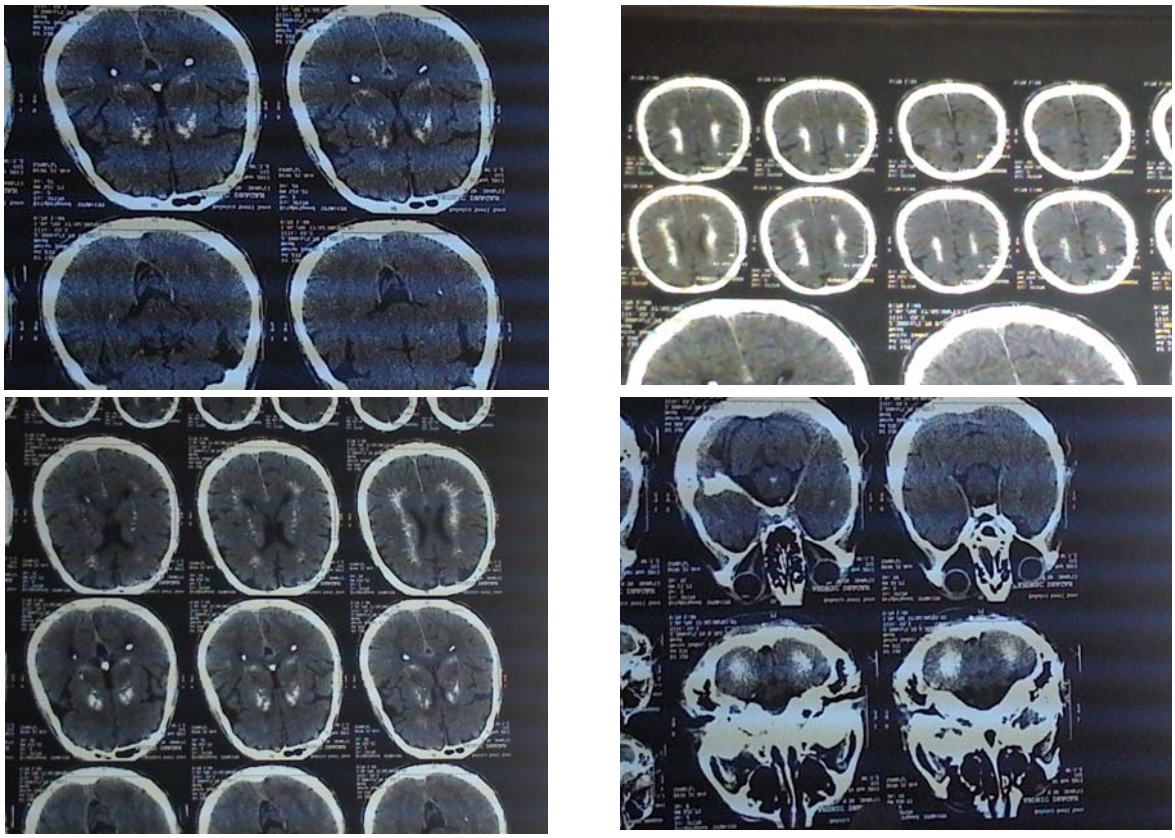

Fig. 2 − Brain computed tomography (CT) scan revealing bilateral, multiple, symmetric calcifications in the basal ganglia, periventricular and supraventricular, also infratentorial in the cerebellum and pons centrally.

On the basis of clinico-radiological and biochemical findings, diagnosis of primary HP and Fahr's syndrome was suggested.

Immediately after admission to the clinic, the patient got intravenous rehydration, antibiotics and calcium infusion, followed by oral supplemental calcium and calcitriol. Parenteral anticoagulation therapy was also conducted, while thiazide diuretics were administered in order to diminish calciuria. Dominant neuropsychiatric signs were stopped by mild anxiolytic therapy.

Gradually after two weeks, all laboratory tests, clinical signs, and electrocardiogram finding went back to normal (Figure 3). He was recommended to take lifelong calcium and calcitriol substitution. Ambulatory, a 6-month control, evidenced maintaining of good general condition without any neurological symptoms or mental disorder. Serum calcium and ionized calcium levels were held at the lower reference limits.

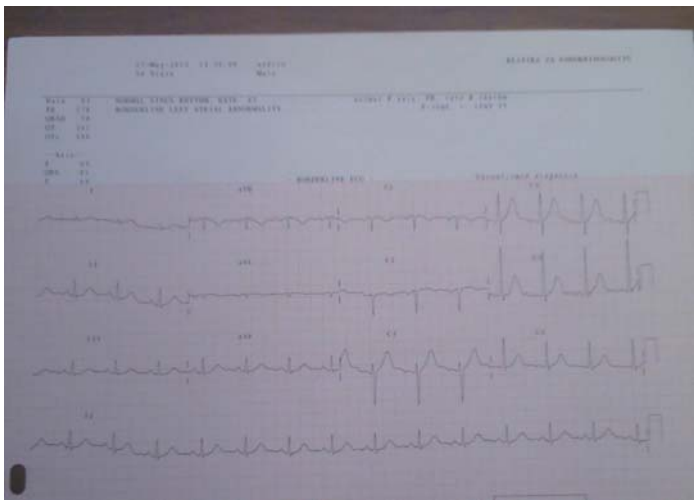

Fig. 3 − Control electrocardiogram evidencing normalization of the duration of electrical systole and out of the first degree atrioventricular block.

Discussion

Basal ganglia calcification (BGC) is a nonspecific finding in 1% of radiographic brain examination [13, 14]. These calcifications could be conditionally separated in two main forms: primary and secondary [3].

The term Fahr's disease refers to cases of idiopathic calcifications in the basal ganglia and other brain regions, and is clinically defined as bilateral BGC in the presence of neuropsychiatric and extrapyramidal disorders with normal calcium and parathyroid levels [4, 12]. Fahr's disease could be sporadic or inherited in an autosomal dominant pattern with the commonly involved locus at 14 q chromosome. Some form of this disease may also be passed on as an autosomal recessive trait [1, 4, 5, 15, 16]. This disease is also known as familial idiopathic basal ganglia calcifications; still there are 35 additional terms used for this condition in the literature. Some synonyms for this disease are: idiopathic striopallidodentate calcinosis, cerebrovascular ferro calcinosis, calcinosis nucleorum, familial idiopathic cerebral calcifications etc. [3, 9].

In contrast to this primary form, bilateral and symmetric calcifications of basal ganglia and other brain regions, can occur secondary, as the consequence of various metabolic, infectious, or degenerative disease. These includes endocrine disorders, mitochondrial myopathy, some dermatological disorders, brucellosis, toxoplasmosis, etc. [3, 4, 13]. This condition is known as Fahr's syndrome. Clinically, it should be clearly distinguished from Fahr's disease [3].

Therefore, common for both conditions are bilateral BGC and progressive neuropsychiatric manifestations. The essential difference between the disease and the syndrome is the presence of the family history of BGC (Fahr's disease) and evidence of some other, known cause (Fahr's syndrome).

The most common reported metabolic disorders that cause Fahr's syndrome are HP and pseudohypoparathyroidism [1, 17]. HP could be iatrogenic, as the consequence of surgical removal or radiotherapy, or could be idiopathic [1, 6, 13]. The latter refers to deficient PTH secretion without a defined cause and includes a group of rare conditions that can be genetically inherited and/or autoimmune. Inherited genetic HP may run in families by passing on with autosomal dominant, autosomal recessive or X-linked pattern, and may occur in childhood or later in life. Autoimmune HP may be isolated, or exist as a part of polyglandular syndrome such as autoimmune polyglandular syndrome type 1 or type 2. The former disorder is also inherited, but it usually occurs until the period of early adolescence and always by the age of 25. The latter presents in adulthood in combination with adrenal insufficiency, type 1 diabetes mellitus, or thyroid autoimmune diseases [5–7, 13, 18]. The most common cause of HP is damage or removal of the parathyroid glands during thyroidectomy [19, 20]. In postoperative HP, basal ganglia calcifications will develop in untreated patients, after a median of 17 years [13, 21]. Early treatment of postoperative HP can prevent brain calcifications with typical manifestations [6, 10]. In patients with idiopathic form of HP, adequate oral calcitriol and calcium supplementation is needed in order to restore calcium/phosphorus ratio and reduce the risk of basal ganglia calcification appearance and progression [6, 13, 22].

There is no clear explanation for the mechanism of brain calcification and hypocalcemia association. It is suggested that increased calcium-phosphorus complex formation plays an important role [6, 13]. It is possible that the subsidence of calcium in the brain parenchyma, appear due to local disruption of the blood-brain barrier or due to a disorder of neuronal calcium metabolism. Active role of PTH in the basal ganglia physiology could also be involved. There are some findings that psychiatric conditions may be associated with calcium dysregulation, calcium signaling and altered calcium homeostasis [3, 4]. Histological examination of affected areas revealed concentric calcium deposits within small and medium-sized arterial walls as well as droplet calcifications along capillaries [6].

The most common clinical features are neurological as seizures, spasticity, choreoathetosis, tremor, headache, vertigo, dysarthria, loss of consciousness. Psychiatric features include depression, manic symtoms, irritability, aggression, or deterioration of intelligence [1, 3, 4, 16, 18]. The impact of changes in calcium levels in the QT interval in ECG recording is well-known. In our case, electrocardiogram revealed episodes of sinus tachycardia with first degree AV block and prolonged depolarisation, fully retreated with normal serum calcium levels. Cardiac conduction disease were also observed in some cases of FD [23].

In contrast to typical slowly progressive neuropsychiatric manifestations, in our case, all of the neurological symptoms occurred suddenly, and were provoked by respiratory infection. Polymorphic symptoms, including diffuse cramps, diarrhea, and body pain were non specific, while mental disorientation and irritability were noticed only periodically. Other neurological manifestations, such as ataxia, adynamia, slurred speech, and mental confusion progressed rapidly in a few days. All of the symptoms related to hypocalcemia occurred in a short, 2-month interval, with no accompanied endocrine disorder, suggesting isolated idiopathic HP. Still, these symptoms were also the most prominent feature of clinical presentation in our patient, with positive Trousseau sign, decreased serum calcium, and PTH levels, followed by typical prolonged QT interval on electrocardiographic (ECG) examination. CT finding of bilateral brain calcifications completed the diagnosis towards Fahr's syndrome due to HP.

To date, there is no standard course of treatment for Fahr's syndrome/disease. A recent prospective study has found that increased risk for BGC progression is significantly associated with low calcium/phosphorus ratio, hyperphosphatemia and history of seizures [6, 13]. Nevertheless, serum levels of 25(OH)D vitamin and 1.25 (OH)2D vitamin were not significantly associated with progression. Interestingly, with every 1% increase in the calcium/phosphorus ratio, progression of basal ganglia calcification decreased by 5% [6, 13, 19].

Recommended treatment is directed toward symptomatic control of neurological manifestations using anxiolytics, antipsychotics, anticonvulsant treatment with appropriate rehydration, electrolyte, and hemodynamic balance maintenance [4, 8, 18]. In cases of Fahr's syndrome due to hypoparathyroidism, the neurological and psychiatric

symptoms usually improve with normalisation of plasma calcium and phosphorus levels. It is recommended to obtain a target serum calcium level in the low normal range [6, 12]. HP is a rare endocrine condition with a hormone deficiency, that does not necessarily require the same substitution [18]. The subcutaneous application of synthetic PTH analogs are recommended only in refractory forms with chronic hypercalciuria and kidney complications [5, 7, 13, 24, 25].

The presented patient showed no need for serious anticonvulsant or antipsychotic treatment. Dramatic clinical presentation and difficult general state of the presented patient was fully withdrawn after only a few days of intravenous rehydration and calcium substitution, followed with thiazide diuretics that reduced urinary loss of calcium. After achieving rapid resolution of symptoms, his treatment continued with oral supplemental calcium and calcitriol preparations for maintenance of serum calcium in the low to normal range.

Conclusion

Although rare, Fahr's syndrome or/and Fahr's disease are conditions that should be keep in mind in all the cases of progressive neuropsychiatric disturbances and seizure disorders, particularly if they are present in the fourth or fifth decade of age. In such cases, it is especially important to perform laboratory tests for the purpose of prompt registration of possible metabolic abnormalities. Any suspected hypoparathyroidism should be treated in time, especially in subjects that underwent thyroidectomy in order to prevent formation and progression of brain calcifications. On the other hand, every intracranial calcification, incidentally detected during radiographic imaging, needs thorough neurological examination and biochemical tests. Timely recognition of these two entities allows appropriate treatment that can prevent clinical manifestations of the disease and potentially slow its progression.

R E F E R E N C E S

1. *Saleem S, Aslam HM, Anwar M, Anwar S, Saleem M, Saleem A*, et al. Fahr's syndrome: Literature review of current evidence. Orph J Rare Dis 2013; 8(1): 156.
2. *Asokan AG, D'Souza S, Jeganathan J, Pai S*. Fahr's Syndrome-An Interesting Case Presentation. J Clin Diagn Res 2013; 7(3): 532−3.
3. *Kavirayani DK, Jammulapati S*. Fahr's syndrome with psychotic features: Review and Case report. NMJ 2014; 3(2): 71−4.
4. *Kumar S, Sher K, Ahmed S, Naik S, Ayub S, Fatima BM*, et al. Fahr's Disease: A Rare Neurological Disease Frequently Misdiagnosed as Acute Psychosis, or Mood Disorder. J Neurol Disord 2013; 1: 130.
5. *Shoback D*. Clinical practice. Hypoparathyroidism. N Engl J Med 2008; 359(4): 391−403.
6. *Goswami R, Sharma R, Sreenivas V, Gupta N, Ganapathy A, Das S*. Prevalence and progression of basal ganglia calcification and its pathogenic mechanism in patients with idiopathic hypoparathyroidism. Clin Endocrinol (Oxf) 2012; 77(2): 200−6.
7. *Mitchell DM, Regan S, Cooley MR, Lauter KB, Vrla MC, Becker CB,* et al. Long-term follow-up of patients with hypoparathyroidism. J Clin Endocrinol Metab 2012; 97(12): 4507−14.
8. *Caliskan T, Gurbuz S*. Fahr's Disease. J Contemp Med 2013; 3(2): 133−5.
9. *Manyam BV*. What is and what is not 'Fahr's disease'. Parkinsonism Relat Disord 2005; 11(2): 73−80.
10. *Eaton LM, Camp JD, Lore JG*. Symmetric cerebral calcification, particulary of basal ganglia, demonstrable roentgen nographically; calcific ation of the finer cerebral blood ressels. Arch Neurol Psychiatres 1939; 41: 921−42.
11. *Krishnamurthy K, Sonasree J*. Fahr's syndrome with psychotic features – Review and Case report. NMJ 2014; 3(2): 71−4.
12. *Rastogi R, Singh AK, Rastogi UC, Mohan C, Rastogi V*. Fahr's syndrome: A rare clinico-radiologic entity. Med J Armed Forces India 2011; 67: 159−61.
13. *Soares FB, Amorima FF, Santana AR, de Moura EB, Margalho SB, Amorim AP,* et al. Fahr's Syndrome due to Hypoparathyroidism Following Thyroidectomy. J Med Cases 2013; 4(6): 380−4.
14. *Fénelon G, Gray F, Paillard F, Thibierge M, Mahieux F, Guillani A*. A prospective study of patients with CT detected pallidal calcifications. J Neurol Neurosurg Psychiatr 1993; 56(6): 622−5.
15. *Moskowitz MA, Winickoff RN, Heinz ER*. Familial calcification of the basal ganglions: A metabolic and genetic study. N Engl J Med 1971; 285(2): 72−7.
16. *Mufaddel AA, Al-Hassani GA*. Familial idiopathic basal ganglia calcification (Fahr`s disease). Neurosciences (Riyadh) 2014; 19(3): 171−7.
17. *Ahad MA, Bala CS, Karim SR*. Fahr's Syndrome. Bang Med J (Khulna) 2012; 45(1−2): 33−5.
18. *Rizvi I, Ansari NA, Beg M, Shamim MD*. Widespread intracranial calcification, seizures and extrapyramidal manifestations in a case of hypoparathyroidism. N Am J Med Sci 2012; 4(8): 369−72.
19. *Chowdhury AW, Majumder SN, Amin MG, Islam KN, Saleh MA, Sabah KM*, et al. Recurrent Seizure: An Uncommon Presentation of Post Thyroidectomy Hypoparathyroidism. Bangladesh J Vet Med 2015; 16(1): 56−8.
20. *Zisimopoulou V, Siatouni A, Tsoukalos G, Tavernarakis A, Gatzonis S*. Extensive bilateral intracranial calcifications: A case of iatrogenic hypoparathyroidism. Case Rep Med 2013; 2013: 932184.
21. *Castro MC, Singer BH*. Agricultural settlement and soil quality in the Brazilian Amazon. Popul Environ 2012; 34(1): 22−43.
22. *Ghosh K, Sengupta N, Sau TJ, Chatterjee A*. Rare presentation of a common disease: Idiopathic hypoparathyroidism presenting with extrapyramidal symptoms and status epilepticus. Indian J Endocr Metab 2012; 16(6): 1035−6.
23. *Panduranga P, Sulaiman K*. Is there an association between Fahr's disease and cardiac conduction system disease?: A case report. J Res Med Sci 2012; 17(1): 96−100.
24. *Winer KK, Zhang B, Shrader JA, Peterson D, Smith M, Albert PS,* et al. Synthetic human parathyroid hormone 1-34 replacement therapy: a randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. J Clin Endocrinol Metab 2012; 97(2): 391−9.
25. *Winer KK, Cutler GB*. Treatment of Hypoparathyroidism with Parathyroid Hormone 1-34. In: *Brandi ML, Brown EM*, editors. Hypoparathyroidism. Milano: Springer-Verlag Italia; 2015. p. 319−31.

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CASE REPORT

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Fixed prosthetic treatment in patients with cleft lip and palate

Fiksnoproteski tretman pacijenata sa heilognatopalatoshizom

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Abstract

Introduction. The prosthetic treatment of patients with cleft palate includes various treatment options such as fixed partial dentures, removable partial prosthesis, etc. The type of prosthetic appliance is determined by the oral health of each individual and the circumstances. We presented three adult patients with the cleft lip and palate subjected to prosthetic treatment. **Case report.** From the possible prosthetic solutions according to the conditions in the oral cavity and the circumstances, fixed partial dentures veneered with composite or ceramic were chosen. A proper relationship between the teeth was reached with the fixed partial dentures, and function established, the phonetics improved and satisfying aesthetics effect accomplished improving the profile appearance of the patient's face. Plastic surgery of the nose was performed after that. **Conclusion.** Multidisclipinary treatment is necessary for favourable long-term outcome in cleft lip and palate patients.

Key words:
cleft lip; cleft palate; prosthodontics; dental prosthesis; denture, partial, fixed; treatment outcome.

Apstrakt

Uvod. Protetsko lečenje pacijenata sa rascepom nepca uključuje različite opcije kao što su fiksne parcijalne proteze, parcijalne pokretne proteze i tako dalje. Tip protetskog aparata zavisi od oralnog zdravlja svake osobe pojedinačno, kao i od okolnosti. Prikazana su tri odrasla pacijenta sa heilognatopalatoshizom koji su zbrinuti protetski. **Prikaz bolesnika.** Od mogućih protetskih rešenja, u zavisnosti od uslova u usnoj duplji, izabrano je da se urade mostovne konstrukcije, fasetirane sa kompozitom ili keramikom. Sa mostovnom konstrukcijom uspostavljeni su međusobni odnosi zuba, postignuta je pravilna funkcija, poboljašala se fonetika, omogućena je zadovoljavajuća estetika i postignut je zadovoljavajući profilni izgled lica pacijenata. Usledila je plastična operacija nosa. **Zaključak.** Za povoljan dugoročni ishod lečenja bolesnika sa rascepom nepca i usne neophodan je multidisciplinarni pristup.

Ključne reči:
usna, rascep; nepce, rascep; protetika; zubna proteza; zubna proteza, parcijalna, fiksna; lečenje, ishod.

Introduction

The occurrence of congenital cleft lip and palate has several possible etiological factors. As possible causes stated in the literature are deficient diet and psychological stress during pregnancy, chemical teratogenic agents, infectious diseases (viral origin), radiation during pregnancy and hereditary factors, gene mutation or chromosome aberration. The increased incidence of cleft lip and palate was observed during the periods of war as compared with peaceful times [1]. For the treatment of patients with cleft lip and palate a multidisciplinary approach is required including maxillofacial and oral surgeon, orthodontist, prosthodontist, speech therapist, sociologist, pediatrician, and psychologist.

The prosthetic treatment in patients who had cleft palate includes various treatment options such as fixed partial dentures, removable partial prosthesis, overdentures, complete dentures and implant-supported prosthetic dentures [2].

We presented three cases of managing cleft lip and palate in adult patients with fixed partial dentures.

Case report

Three adult patients with cleft lip and palate were referred to the Department of Prosthetics. They all had undergone lip and palate surgery at an early age, one of them was treated orthodontically and the other two patients were mentally disabled and had no orthodontic therapy. The first patient

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and the parents of the other two declined the other suggested treatment modalities and requested fixed prosthetics therapy. The patients had visible scar tissue on the lip and in the cleft area, concave face profile and collapsed nose.

For proper analysis and treatment planning a complete history was taken from the patients and clinical examination was carried out assessing the overall state of the oral cavity (missing teeth, alignment of the present teeth, intraoral deformities, relationship of the alveolar arches in occlusion and the occlusion itself, vertical dimension, oral hygiene, gingival inflammation, length of the clinical crowns, and their ability to serve as abutment teeth for fixed partial dentures). Paraclinical tests were also conducted (pulp testing, x-ray). After performing periodontal treatment in order to obtain healthy supportive tissues, impressions were taken and diagnostic models were poured in dental stone, and were mounted in semiadjustable articulator in order to complete the analysis and information gathered previously. We evaluated the relationship of the alveolar arches and the occlusogingival relation, and diagnostic preparation and modeling in wax was made to determine the size and shape of the future prosthetic restoration. The present malpositioned teeth with insufficient length of radixes and peg-shaped crowns were extracted.

The remaining teeth in the frontal region which were properly aligned and shaped were prepared according to the biomechanical principles and impressions were made using putty/wash silicon impression materials in order to fabricate fixed partial dentures made from metal framework veneered with composite or ceramic.

Case 1

A 18-year-old patient had missing teeth in the frontal region and ectopic teeth scattered on the palate (Figures 1 a and b). The ectopic teeth were extracted. After careful planning a fixed partial dentures were made using precious alloy veneered with composite (Figure 1 c).

Case 2

A 18-year-old patient had missing four incisors, the first left premolar, a part of the alveolar arch in the maxilla (Figures 2 a and b). With the previous orthodontic treatment grupes *tet-a-tet* relationship of the upper and lower teeth was reached. Fixed partial denture mas made from precious alloy veneered with composite and the missing part of the alveolar arch was restored with acrylic (Figures 2 c and d).

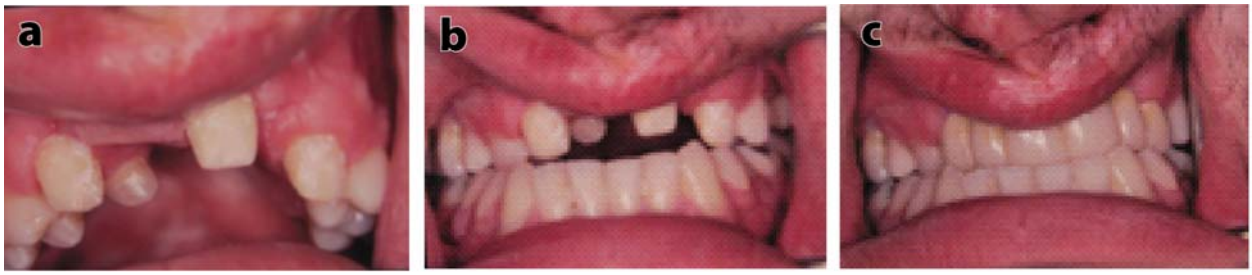

Fig. 1 – a) Dental status in the oral cavity and the maxilla; b) The dental arches in maximal intercuspation; c) Mounted fixed partial denture.

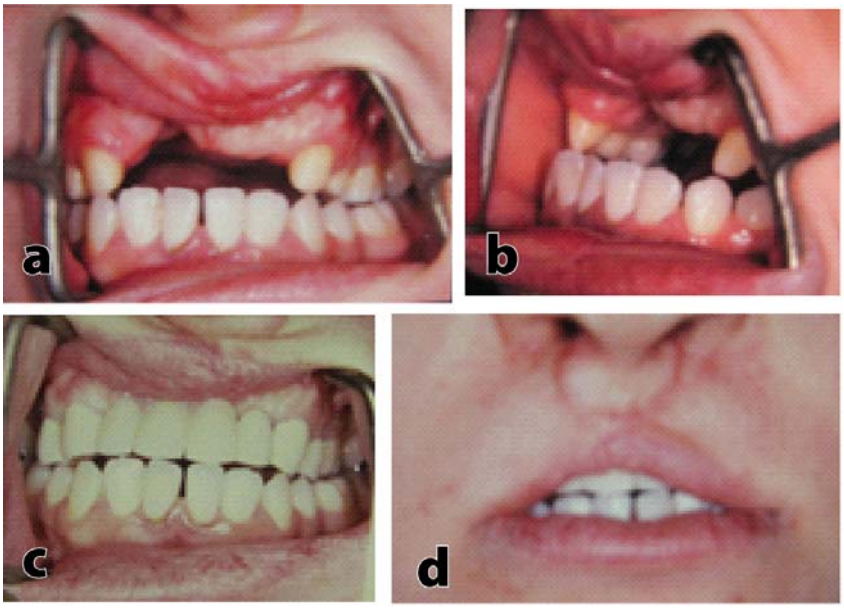

Fig. 2 – a) and b) Condition in the patient's oral cavity; c) Patient with completed prosthodontic restoration; d) Patient appearance.

Case 3

A 18-year-old patient with no orthodontic treatment had scars on the outer and inner part of the lip after the oral surgery. The intraoral status showed micromaxilla and pseudoprogenia, missing and malpositioned teeth, central incisors that were rotated and rudimentary, gingival inflammation and crossed bite in the posterior region (Figures 3 a–c). Because of the skeletal mismatch of the maxilla and mandible there was discrepancy between the alveolar arches. After careful planning, the improperly aligned and rudimentary teeth were extracted and periodontal treatment carried out. The existing vertical dimension was used for fabrication of fixed partial denture from metal-ceramic. The teeth were prepared, and an impression was taken for the dental laboratory. With the finished fixed partial denture, mounted in the patient mouth, the function improved, the upper lip lifted taking teeth-supported position and the aesthetics of the patients face was improved (Figures 3 d–f). Prosthetic treatment was followed with plastic surgery on the nose. Since the patient had low smile line and the gingival third of the fixed partial denture was not visible the aesthetic appearance of the restoration was improved.

Discussion

In the treatment of patients with cleft lip and palate significant attention is given to the analysis of success of so far used protocols of treatment which cover several treatment stages: presurgical orthodontic therapy, plastic surgery of the cleft lip and palate followed with orthodontic therapy and plastic surgery on the nose and scar tissue after completed growth [3].

Restoring the teeth in such cases is demanding and complex task having the pseudoprogenia, crossed bite, hipodontia or hyperdontia in the cleft area with ectopical placement of the teeth, open bite, crowded teeth or teeth with diastemas and lost middle line, and normal or reduced vertical dimension [4].

Several authors presented prosthetic treatment of such cases [2, 5–7]. According to the oral health of each individual and the circumstances, the type of prosthetic appliance is determined [8]. Fixed partial dentures provide comfort, improve speech, mastication and aesthetics influencing positively the overall psychological state of the patient [2].

The literature shows that combined prosthetic modalities are the most frequently used type of prosthetic constructions [9]. Several authors presented treatment approaches in their

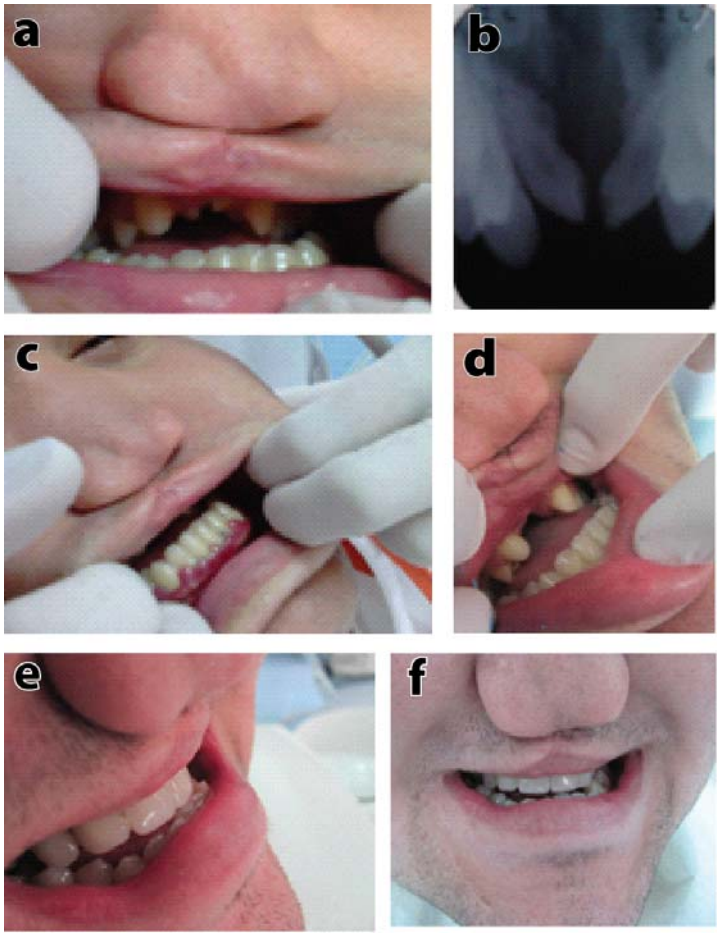

Fig. 3 –a) Micromaxilla; b) X-ray showing the malpositioned teeth; c) Gingival inflammation; d) Abutment teeth after preparation; e) Metal-ceramic fixed partial denture seated in the patient mouth; f) Appearance after prosthodontic rehabilitation.

case reports on cleft lip and palate patients using root copings, modified Dolder bar attachment, conus crown system, modified crowns and removable partial dentures [5], overlay dentures retained with microextracoronar resilient attachments [10] and after obtaining stabile occlusion with orthodontic treatment a fixed partial dentures veneered with composite [11].

Patients with no bone grafting and orthodontic treatment present greatest challenge [12]. Since patients with cleft palate require long time follow-up and maintenance, in case of failure of restoration review of the treatment is still needed and incorporation of superior materials and methods in order to minimize further complications [11].

Conclusion

We presented solutions with fixed prosthetic treatment in patients with cleft lip and palate. With the fixed partial dentures a proper relation between the teeth was reached, function enabled, phonetics significantly improved and satisfying aesthetic overall appearance of the patient accomplished.

R E F E R E N C E S

1. *Meštrovic MM, Bagatin M, Poje Z.* The incidence of orofacial clepts in Croatia in 1988−1998. Acta Stomatol Croat 2005; 39(1): 53−60. (Croatian)
2. *Lopes JF, Pinto JH, de Almeida AL, Lopes MM, da Silva Dalben G.* Cleft palate obturation with Brånemark protocol implant-supported fixed denture and removable obturator. Cleft Palate Craniofac J 2010; 47(2): 211−5.
3. *Vukić-Čulafić B, Vučinić P, Ivić S, Petrović Đ.* The relationship of the parameters of jaw and cranial bases in cheilognathopalatoschisis. Proceedings of the XLVI International Congress of Anthropological Society of Yugoslavia; 2007 May 29 − June 2; Apatin, Serbia. Novi Sad: Glasnik Antropološkog društva Srbije 2008; 43: 140−7. (Serbian)
4. *Suvin M, Kosovel Z.* Fixed prosthodontics. Zagreb: Školska knjiga; 1975. (Croatian)
5. *Vojvodić D, Jerolimov V, Jokić DV, Lončar A.* Oral Rehabilitation of the Patient with Cheilognathopalatoschisis. Acta Stomatol Croat 2000; 34(3): 329−30. (Croatian)
6. *Ayna E, Başaran EG, Beydemir K.* Prosthodontic Rehabilitation Alternative of Patients with Cleft Lip and Palate (CLP): Two Cases Report. Int J Dent 2009; 2009: 515790.
7. *Watanabe I, Kurtz KS, Watanabe E, Yamada M, Yoshida N, Miller AW.* Multi-unit fixed partial denture for a bilateral cleft palate patient: a clinical report. J Oral Rehabil 2005; 32(8): 620−2.
8. *Freitas JA, Almeida AL, Soares S, Neves LT, Garib DG, Trindade-Suedam IK*, et al. Rehabilitative treatment of cleft lip and palate: experience of the Hospital for Rehabilitation of Craniofacial Anomalies/USP (HRAC/USP) - Part 4: oral rehabilitation. J Appl Oral Sci 2013; 21(3): 284−92.
9. *Kranjčić J, Žabarović D, Čelebić A, Mehulić K, Komar D, Vojvodić D.* Prosthetic Modalities Used to Treat Cleft Palate Patients in a University Clinic. Year Review. Coll Antropol 2013; 37(2): 423−9.
10. *Acharya V, Brecht LE.* Conventional prosthodontic management of partial edentulism with a resilient attachment-retained overdenture in a patient with a cleft lip and palate: a clinical report. J Prosthet Dent 2014; 112(2): 117−21.
11. *Bidra AS.* Esthetic and functional rehabilitation of a bilateral cleft palate patient with fixed prosthodontic therapy. J Esthet Restor Dent 2012; 24(4): 236−44.
12. *Moore D, McCord JF.* Prosthetic dentistry and the unilateral cleft lip and palate patient. The last 30 years. A review of the prosthodontic literature in respect of treatment options. Eur J Prosthodont Restor Dent 2004; 12(2): 70−4.

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Periodontology – the historical outline from ancient times until the 20th century

Istorijski razvoj parodontologije

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Introduction

The diseases of the periodontium are considered as old as the recorded history of mankind [1–3]. The historical evaluation of pathology and therapeutics can be traced through the variety of sources: anatomical findings from more or less well-preserved skeletal parts, detailes observed in mummies, instruments and equipments collected during archaelogical investigations and evidence from engravings and various manuscripts [2]. Studies in paleopathology have indicated that a destructive periodontal disease, as evidenced by bone loss, accompanied early human beings in diverse cultures [1–6]. Almost all early historical records that involve dental topics have several chapters dealing with periodontal disease and the need for treatment.

The development of dentistry can be conveniently divided into three periods [2]: magico-religious medicine (5000–400 BC); empirico-rational medicine (400 BC–1500 AD) and scientific medicine (1500 AD – until today).

Magico-religious medicine

Early civilizations

The three oldest civilizations were the Sumerians, the Babylonians and the Assyrians [1–3]. In these early societies with strong and pervasive religion, the cure of any disease depended on driving out the demons thought to cause that particular disease. A Sumerian text from 5000 years BC describes that apparently Sumerians were suffering from periodontal disease [1–3]. They practiced oral hygiene, including gingival massage in combination with various herbal medications [1–3]. This finding was further confirmed by decorated gold toothpicks founded in the exavations at the Nigel Temple, Ur in Mesopotamia [2].

Almost all of our knowledge of Babylonian and Assyrian medicine comes from the clay tablets of the great library of Ashurbanipal (king of Assyria), that includes a number of remedies for periodontal disease, such as "if a man's teeth are loose and itch a mixture of myrrh, asafetida and opopanax, as well as pine-turpentine shall be rubbed on his teeth until blood comes forth and he shall recover" [2].

Many Egyptian medical papyri (Ebers Papyrus, Kahun Papyri, Brugsch Papyrus and Hearst Papyrus) preserved in the museums of Europe provide several details about medical herbs and adjuvants, such as milk, honey, mint, salt and beer, on the one hand and on the other magical invocations, amulets and other curative devices as remedies for many diseases, including periodontal disease, such as "one part each of powder of the friut of palm, green lead and honey to be mixed and the teeth rubbed with it" [1–6]. Radiographs of Egyptian pharaon mummies confirmed that they suffered from periodontal disease [1–6]. A specimen from Cizeh (2500 BC) shows two molars fastened with heavy gold wire, serving as an evidence that Egyptian practiced splinting of the loose teeth [1, 7, 8].

Hesy-Re (2686–2613 BC) was an Egyptian scribe, who lived in the 3rd Egypt Dinasty under pharaon Djoser (Figure 1). He is often called the first "dentist" ("the greatest of the teeth") and this is the earliest identification of a person as a dental practitioner [1, 2]. An inscription on his tomb includes the title "the greatest of those who deal with teeth, and of physicians". He has also been credited as being the first man to recognize periodontal disease [1, 7, 8].

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Fig. 1 – Relief of Hesy-Re [9]

Periodontal disease was also discussed in ancient Indian and Chinese books [1–3, 6, 10]. An ancient Indian book written by Susruta (6th century BC), entitled Susruta Samhita, contains four descriptions of periodontal disease, such as "the gums of the teeth suddenly bleed and become putrefied, black and slimy and emit fetid smell". It is believed that this is the most probably the first classification of periodontal diseases [6]. A later book, Charaka Samhita, discusses proper oral hygiene and toothbrushing: "The stick for brushing the teeth should be either adsringent or pungent or bitter. One of its ends should be chewed in the form of a brush. It should be used twice a day, taking care that the gums will not be injured" [1, 11]. The oldest Chinese book written by Huang-ti (2500 BC) entitled Huang-ti Nei Ching (The Canon of Internal Medicine) describes various conditions affecting oral cavity, including periodontal disease (detailed description of gingival inflammation, periodontal abscess and gingival ulceration). At least seven remedies for periodontal disease are listed in it. He dedicated a significant part of the book to oral hygiene and to date, it is believed that Chinese were among the first people to use the toothbrush and toothpick to clean the teeth [1–3, 6].

The early Hebrews also recognized the importance of oral hygiene. Many pathologic conditions of the teeth and their surrounding structures are described in the Talmud (325–407 AD). Jewish medical practices were also with the attitude that a physician did not really cure a disease, but rather prepared the ground for nature, which was the actual healer. As for periodontal disease it was mentioned "...to start in the mouth but end in the gut" [3]. The Hebrew *materia medica* for periodontal disease was relatively primitive but pepper, salt, ginger and cannel were used to calm dental pain and halitosis [1, 11].

The Greeks

The lives of the ancient Greeks were dominated by Gods and they believed that ilnesses were divine punishments and that healing was a gift from the Gods. By the 5th century BC there were attepmts to explain natural rather than spiritual causses of ilness and Greek medical practitioners began to take greater interest in the human body. They were constantly developing in all areas, including trade, sailing, craftmanship, as well as science and culture. Their medicine developed accordingly, making a unique contribution in the development of modern scientific medicine [1, 12].

Hippocrates of Cos (460–377 BC) is considered the father of modern medicine, since he separated Greek medicine from superstitions, magic and religion (Figure 2). According to Hippocrates, health of the body was defined as a balance in between four humours – blood, phlegm, yellow bile and black bile. When these humours are in dispropportion, the disease will occur. He wrote *Corpus Hippocraticum* (The Hippocratic Collection) devoting 32 paragraphs to dentition [11]. In his work he discussed the function and eruption of the teeth and the etiology of periodontal disease. He believed that inflammation of the gums could be caused by accumulations of "pituita" or calculus, with gingival hemorrhage occurring in cases of persistent splenic maladies. One splenic malady was described as: "The belly become swollen, the spleen enlarged and hard, the patient suffer from acute pain. The gums are dettached from the teeth and smell bad" [1, 11].

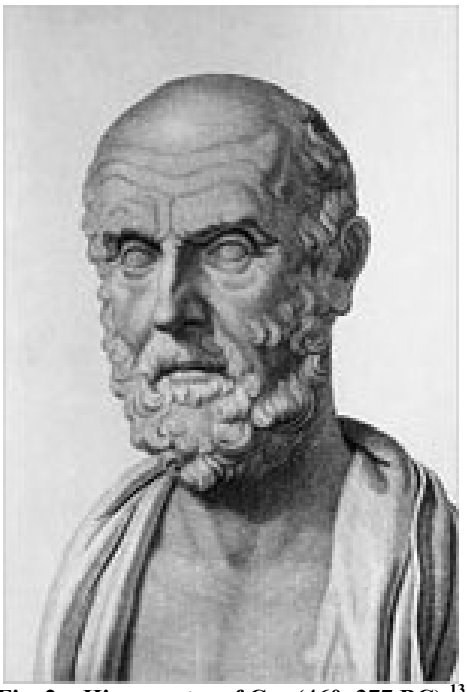

Fig. 2 – Hippocrates of Cos (460–377 BC) [13]

The Romans

Roman empire was one of the largest and most powerful empires in ancient history. Ancient Roman scientists and doctors were under the influence of ancient Greeks, and they continued researching Greek theory of diseases (fo-

ur humors). They were particulary interested in dental prevention (oral hygiene) rather than cure. Therefore, it is not a surprise that the use of the toothbrush is mentioned in many Roman poems [1–3].

Aulus Cornelius Celsus (25 BC – 50 AD) wrote *De Medicina*, extensively talking about diseases that affect the soft parts of the mouth and their treatment as: "If the gums separate from the teeth, it is beneficial to chew purslane or pears and apples and keep their juices in the mouth" [1–3]. His book also contains important information about oral hygiene and stabilization of loose teeth.

Galen of Pergamon (129–200/216 AD) the doctor of the Roman Emperor Marcus Aurelius wrote the first article about dentistry. According to Galen, periodontal disease is caused by "relaxation of the dental nerve due to excessive abundance of humors" [1]. Galen greatly influenced European medicine for several centuries.

Empirico-rational medicine

The decline of Roman Empire plunged Europe into the ages of darkness. This was a period of the expansion of Islam in Europe and golden age of Arabic science and medicine. Arabic physicians were mainly influenced by translated ancient Greeks' medical treatises (from Hippocrates and Galen) and the elements from Indian and Hebrew medicine. This period was characterized with the systematic novel approaches and refinements in techniques, mainly in surgical specialties [1–3]. Empirico-rational medicine was characterized by observation and experimentation rather than influence of magic and religion. It greatly influenced future medieval and reneissance dentistry [1–3].

Paul of Aegina/Paulus Aegineta (625–690) wrote *Epitomoe medicoe libri septem* (Medical Compendium in Seven Books) where he described that tartar deposits must be removed with either scrapers or a small file and that the teeth should be carefully cleaned after the last meal of the day [14].

Abu al-Qasim, also known as Albucasis (936–1013) was Spanish-Arabian physician. His 30-volume encyclopedia *Kitab al-Tasrif* (The Method of Medicine) is the medical text that contains illustrations of dental instruments with detailed description of its use (Figure 3).

Albucasis had a clear understanding of the major etiologic role of calculus deposits, writting: "sometimes on the surface of the teeth, both inside and outside, as well as under the gums, are deposited rough scales of ugly appearance and black, green or yellowish in color; thus corruption is communicated to the gums and so the teeth are in process of time denuded". He invented and proposed the use of many elevators and scalers, described the techniques of scaling the teeth and splinting loose teeth with gold wire [1–3, 15].

Ibn Sina, also known as Avicenna (980–1037 AD) was possibly the greatest of the Arabic physicians (Figure 4). His 14-volume *Al-Qanoon fi al-Tibb* (The Canon of Medicine) was in continuous use for almost 600 years. Avicenna used an extensive *materia medica* for oral and periodontal diseases. His book discusses bleeding gums, fissures and ulcers of the gums, separation, recession and looseness of gums and epulis [1, 2].

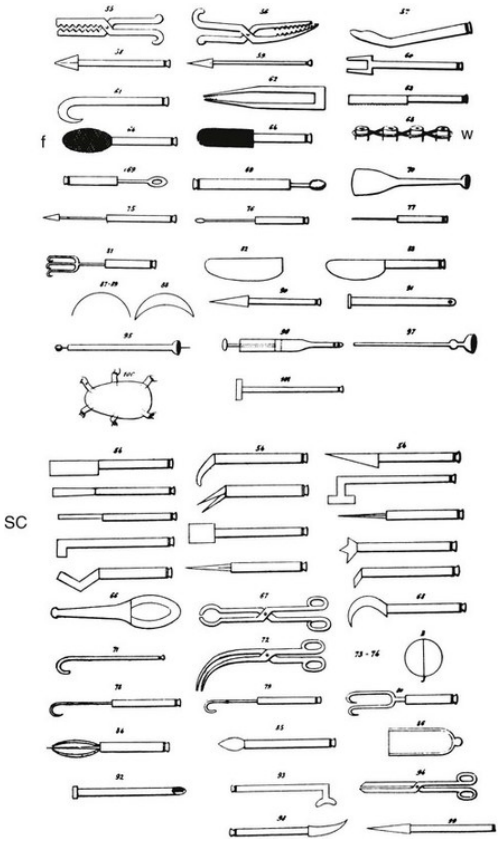

Fig. 3 – Illustration of Albucasis' periodontal instruments [16]

Fig. 4 – Avicenna (980–1037 AD) [17].

Guy de Chauliac (1290–1368) was a French physician and surgeon who wrote his celebrated 7-volume book *Chirurgia Magna* (The Great Surgery). He invented the dental pelican (in the 20th century replaced by forceps) and coined the term "dentators". According to de Chauliac, loose teeth are the result of different causes, such as "humidity which softens the nerve and ligament; dryness and lack of nourishment of the teeth and corrosion of the gums" [1, 18].

Serefeddin Sabuncuoglu (1385–1468) was the Turkish surgeon who wrote *Cerrahiyyetu'l-Haniyye* (The Imperial Surgery). In his book he basically expanded Albucasis' works by illustrations of the surgical removal of hypertrophic and swollen gingiva and lingual frenum. He suggested that "...drug treatment should be initiated if there are swollen gums, mobile teeth and pus formation present. If there is no

response, later surgical treatment should be performed with a tube placed on the gums. Gingival tissue cauterization is performed by hot cautery, inserted into the cannula" [1–3, 19].

Scientific medicine

Renaissance

The Renaissance was a great period in European history, during which there was an intellectual revival in the ideas of ancient Rome and Greece and artistic development. Regarding dentistry, the focus of treatments shifted from a divinely ordained natural balance towards a more scientific approach. Knowledge advanced through the scientific method, such as conducting experiments, collecting observations and reaching conclusions. Since printing press was invented, medical/dental ideas were printed in books that spreaded around Europe easily. The roots of scientific medicine were set.

Leonardo da Vinci (1452–1519) was an anatomist and original dissector of the human body. His manuscript presents the earliest accurate drawings of the teeth and associated structures [1].

Paracelsus (1493–1591) developed an interesting and unusual theory of disease: "The Doctrine of Calculus". Paracelsus recognized the extensive formation of tartar on the teeth and related this to toothache. He considered toothache to be comparable to pain produced by calculus in other organs, such as the kidneys [2].

Girolamo Cardano (1501–1576) was the Italian physician and the first to differentiate the types of periodontal disease [1, 14]. In his publication *De Dentibus* (About the Teeth), he mentioned a type of disease that occured with the advancing age and led to progressive loosening and the loss of teeth, as well as a second very aggressive type that occured in younger patients. In the 20th century Cardano's classification was rediscovered, modified and became widely accepted [20].

Ambroise Paré (1510–1590), French military head surgeon, is known as the "Father of Surgery" (Figure 5).

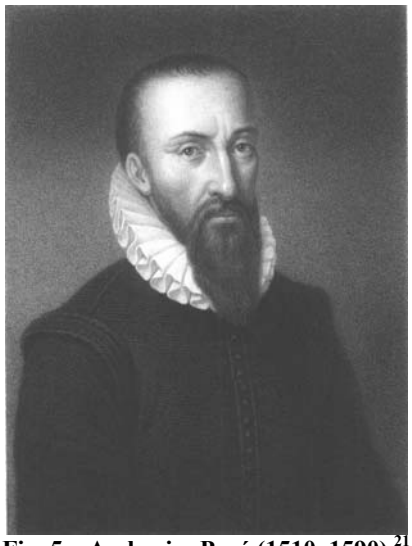

Fig. 5 – Ambroise Paré (1510–1590) [21]

Paré introduced the lancing of infants' gums using a lancet during teething. This belief and practice persisted until the end of the 19th century, when lancing was abandoned. He understood the etiologic significance of calculus and used a set of scalers to remove the hard deposits on the teeth. He developed many oral surgical procedures, such as gingivectomy for hyperplastic gingival tissues [1–3, 22].

Andreas Vesalius (1513–1564) wrote a book *De Humani Corporis Fabrica/Libri Septum* (Fabric of the Human Body/Seventh Book) about teeth development and anatomy that included many excellent illustrations [1, 2].

Bartholomeus Eustachius/Bartolomeo Eustachi (1520–1574) wrote a 30-chapter book *Libellus de Dentibus* (A Little Treatise on the Teeth). This is the first original book about the teeth describing the accurate anatomy of the teeth and the phenomena of the first and second dentition (Figure 6).

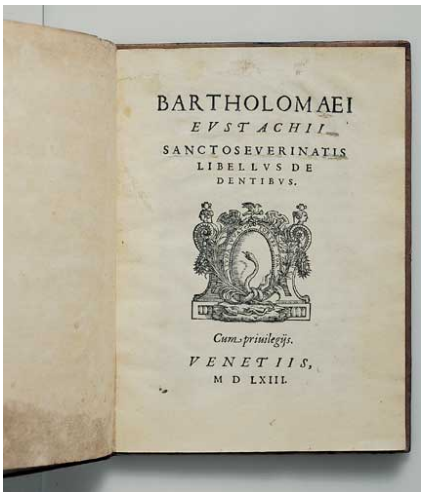

Fig. 6 – *Libellus de Dentibus*, written by Bartholomeus Eustachius [23]

It also includes a description of the periodontal tissues, as well as information about the diseases of the mouth, their treatment modalities and the rationale for treatment. Concering the treatment of periodontitis, Eustachius had very modern ideas and recommended both the scaling of calculus and the curettage of granulation tissue to promote reattachment of the gingival and periodontal tissue [1, 24].

The first book focused solely on dental practice and written in common language of German was entitled *Artzney Buchlein* (The Little Medicinal Book for All Kinds of Diseases) or *Zene Artzney* (Medicine of the Teeth). The book contains three chapters devoted to periodontal disease, including a crude concept of systemic and local infective factors associated with its etiology. As treatment remedies, variety of ointments, which are often astringent in nature, are suggested. Further, the binding of loose teeth with silk or gold thread is recommended. Cauterizing the gingiva with a hot iron is mentioned. Up to date, the author of this book remained unknown [1–3].

Anton van Leeuwenhoek (1632–1723) of Holland, was a layman, but he had an inquisitive mind and a hobby of grinding lenses that allowed him to develop the microscope (1673). He used it to discover and describe the microorganisms ("animalcules"), cellular structure, blood cells, sperm, and various other microscopic structures, including the tubular structure of dentin. Using material from his own mouth,

Leeuwenhoek first described oral bacterial flora, and his drawings offered a reasonably good presentation of oral spirochetes and bacilli. He even performed antiplaque experiments involving the use of strong vinegar in his own mouth and in vitro on bacteria in a dish [1–3, 25].

The 18th century

Modern dental profession essentially developed in the 18th century Europe, particulary in England and France. During 18th century the treatises were published, scientific lectures were given, the first surgeons were trained specifically in dentistry, nonsense remedies were rejected and many inventions were patented.

Pierre Fauchard (1678–1761), French surgeon became known as the “Father of modern dentistry” (Figure 7). Fauchard truly metamorphosed the primitive "practice" of dentistry at the time into a new vocation now fully deserving of the term “profession” [1]. His book, *Le Chirurgien Dentiste* (The Surgeon Dentist) covered all aspects of dental practice, including restorative dentistry, prosthodontics, oral surgery, periodontic and orthodontics [26]. Fauchard described in details periodontal instruments he invented (“donkey snout”, “parrot's beak”, “three-faced burin”, “convex-bladed knife” and “Z-shaped hook”). Further, Fauchard described the scaling technique using instruments he invented, in order to „detach hard matter or tartar from the teeth“ and many remedies to „strenghten the gums“. He also suggested immoblization of the loose teeth by golden wire [1, 26]. Even though there is nothing very original in his work regarding periodontal disease, he did have a great merit of presenting certain therapy (scaling and immobilization) and preventive (personal hygiene) concept in authoritative way. Fauchard's book transformed dental practice, inspired and educated the succeeding generation of dentists [1]. The famous are his words: “Should enlightenment grow in the practice of dentistry, we might attain to progress and engender new ideas...”

John Hunter (1728–1793), British surgeon who wrote an excellent treatise on dentistry entitled The Natural History of the Human Teeth and Practical Treatise on the Diseases of the Teeth. In his books he offered remarkably clear illustrations of the anatomy of the teeth and their supporting structures, and he described the features of periodontal diseases. In collaboration with the London-based dentist James Spence, he began to theorise about the possibility of tooth transplantation from one person to another [1–3, 28].

Thomas Berdmore (1740–1785) was known as “Dentist to His Majesty”. In his book Treatise in the Disorders and Deformities of the Teet and Gums he devoted several chapters (mainly Chapter 7) to periodontal problems [1–3].

The 19th century

The 19th century is described as a time of advanced science and education. By 1800 there were still relatively few dentists practicing the profession. By the middle of the 19th century the number of practicing dentists had increased markedly, although there was no legal or professional control to prevent malpractice and incompetence. Pressure for reform of the profession increased.

Leonard Koecker (1785–1850) was a Baltimore dentist. In a Principles of Dental Surgery he mentioned the careful removal of tartar and the need for oral hygiene by the patient, recommending that it should be performed in the morning and after every meal with the use of an astringent powder and a toothbrush, with care taken to place “the bristles … into the spaces of the teeth”. Koecker was an early advocate of the “odontogenic focal infection” theory, and he recommended the extraction of all severely involved teeth and roots, including all unopposed molars, to prevent systemic infections [1–3].

Levi Spear Parmly (1790–1859) was a New Orleans, Louisiana, dentist who is considered the father of oral hygiene and the inventor of dental floss.

John M. Riggs (1811–1885) was the leading authority on periodontal disease and at the time, periodontitis was known as “Riggs’ disease” (Figure 8). Riggs seems to have been the first individual to limit his practice to periodontics

Fig. 7 – Portrait of Pierre Fauchard (1678–1761) [27]

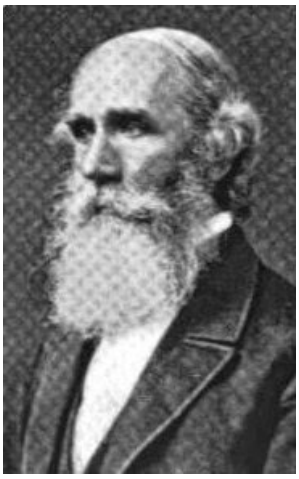

Fig. 8 – John M. Riggs (1811–1885) [29]

and therefore can be considered the first specialist in this field. Riggs' publications contain a strong proponent of the so-called conservative approach to periodontal therapy. He developed the concept of oral prophylaxis and prevention, advocated for the cleanliness of the mouth and opposed surgery, which at the time consisted of gingival resection. Riggs designed a series of six hand instruments, that were not sofisticated and suitable for fine scaling [29]. In 1867 at the meeting of the Connecticut Valley Dental Association, Riggs gave a presentation that was consider fundamental for teaching participants about his periodontal knowledge of his patients. He was follwed by L. Taylor, D. D. Smith, R. B. Adair and W. J. Younger [1–3].

William J. Younger (1838–1920) formulated the possibility of "dento-gingival reattachment" succeeding the postoperative formation of granulation tissue [1]. He designed the scaling instruments which have been the basis for modern instruments used until today [1–3].

Adolf Witzel (1847–1906) was considered first to identify periodontal bacteria, but the first oral microbiologist was Willoughby D. Miller (1853–1907), "Father of dental prevention", who desribed the features of periodontal disease and their contribution in the diseasse development in his classic The Microorganisms of the Human Mouth. He believed that periodontal disease was not caused by one, but many bacterial species present normally in oral cavity ("non-spoecific plaque hypothesis"). Miller did not recognize, nor distinguish oral plaque [2,3].

Leon J. Williams (1852–1932) first described dental plaque as "gelatinous accumulation of the bacteria adherent to the enamel surface" [1,2].

J. H. Vinsent (1862–1950) described the spirillum and fusiform bacilli associated with what later became Vinsent's agina [1–3].

Moritz Karolyi (1865–1945) published an original idea attributing a possible role of dental occlusion in the aetiopathogenesis of periodontal diseases [2].

Edward Kells demonstrated the use of Röentgen x-rays in dentistry in 1896 (the first dental x-ray). This invention, as well as the discovery of anaesthetic dramatically changed the history of dentistry. Horace Wells (1815–1848) use nitrous oxide anaesthesia in 1844, while William Green Morton (1819–1868) used ether in 1846. In 1905 Alfred Einhorn introduced novacaine and adrenalin combination for local anaesthesia, what quickly became a golden standard in local anaesthesia.

Conclusion

What we know today as periodontology bears little or no resemblance to that which was practiced in the early centuries of humanity. Therefore, historical data of previous practices are of tremendous importance for understanding the development of periodontology from ancient to modern times. Developments of previous practices made modern periodontology completely different and far more successful than it was before.

REFERENCES

1. *Shklar G, Carranza F, Williams RC.* The historical background of periodontology. 1st ed. Chicago, IL: Quintessence Pub Co; 2003. p. 23−44.
2. *Held AJ.* Periodontology: From its origins up to 1980: A survey. Basel, Boston, Berlkin: Springer Basel AG; 1989. p. 1−37.
3. *Carranza FA, Newman MG.* Clinical periodontology. 8th ed. Philadelphia, PA: WB Saunders Co; 1996. p. 1−20.
4. *Yilmaz S, Efeoğlu E, Noyan U, Kuru B, Kiliç AR, Kuru L.* The evolution of clinical periodontal therapy. J Marmara Univ Dent Fac 1994; 2(1): 414−23.
5. *Hujoel P, Zina LG, Cunha-Cruz J, Lopez R.* Historical perspectives on theories of periodontal disease etiology. Periodontol 2000 2012; 58(1): 153−60.
6. *Dentino A, Lee S, Mailhot J, Hefti AF.* Principles of periodontology. Periodontol 2000 2013; 61(1): 16−53.
7. *Mitsis FJ, Taramidis G.* Alveolar bone loss on neolithic man remains on 38 skulls of Khirokitia's (Cyprus) inhabitants. J Clin Periodontol 1995; 22(10): 788−93.
8. *Ziskind B, Halioua B.* Occupational medicine in ancient Egypt. Med Hypotheses 2007; 69(4): 942−5.
9. Relief of Hesy-Ra from his Mastaba. Available from : https://commons.wikimedia.org/wiki/File:Hesy-Ra_CG1428_detl.jpg
10. *Loukas M, Lanteri A, Ferrauiola J, Tubbs RS, Maharaja G, Shoja MM,* et al. Anatomy in ancient India: A focus on the Susruta Samhita. J Anat 2010; 217(6): 646−50.
11. *Gurudath G, Vijayakumar KV, Arun R.* Oral Hygiene Practices. Ancient Historical Review. J Orofac Res 2012; 2(4): 225−7.
12. *El-Gammal SY.* The role of Hippocrates in the development and progress of medical sciences. Bull Indian Inst Hist Med Hyderabad 1993; 23(2): 125−36.
13. Hippocrates of Cos (460−377 BC). Available from: https://hankeringforhistory.com/wp-content/uploads/Hippocrates-of.Cos.jpg
14. *Gurunluoglu R, Gurunluoglu A.* Paul of Aegina: Landmark in Surgical Progress. World J Surg 2003; 27(1): 18−25.
15. *Herschfeld JJ.* Dentistry in the writings of Albucasis during the Golden Age of Arabian medicine. Bull Hist Dent 1987; 35(2): 110−4.
16. Illustration of Albucasis' periodontal instruments. Available from: https://pocketdentistry.com/introduction-thehistorical-background-of-periodontology/
17. Avicenna (980−1037 AD). Available from: https//:filosofia.laguia2000.com/wp-content/uploads/2014/08/Avicena-150x214.jpg
18. *Shklar G.* The dental medicine and surgery of Guy de Chauliac with extended excerpts from the Cyrurgia Magna. J Hist Dent 1997; 45(3): 113−9.
19. *Uzel I.* Dental chapters of Serefeddin Sabuncuoglu's (1385-1468?) illustrated surgical book Cerrahiyyetu'l Haniyye. J Hist Dent 1997; 45(3): 107−12.
20. *Eramo S, Delfino D, Confaloni M, De Carolis C.* The 1562 De dentibus by Girolamo Cardano. Acta Med Hist Adriat 2014; 12(1): 27−76. (Italian)
21. Ambroise Paré (1510-1590). Available from: https//: commons.wikimedia.org/wiki/File:Ambroise_Par%C3%A9.jpg
22. *Walters H, Saad MN.* Ambroise Paré on dentistry. Br Dent J 1970; 129(9): 431−6.
23. Libellus de Dentibus, written by Bartholomeus Eustachius. Available from: https//:tooth-stories.com/2012/03/19/ta-prota-odontiatrika-vivlia/
24. *Shklar G, Chernin D.* Eustachio and "Libellus de dentibus", the first book devoted to the structure and function of the teeth. J Hist Dent 2000; 48(1): 25−30.
25. *Bardell D.* The roles of the sense of taste and clean teeth in the discovery of bacteria by Antoni van Leeuwenhoek. Microbiol Rev 1983; 47(1): 121−6.

26. *Maloney WJ, Maloney MP*. Pierre Fauchard: The father of modern dentistry. J Mass Dent Soc 2009; 58(2): 28−9.
27. Portrait of Pierre Fauchard (1678−1761). Available from : https//: commons.wikimedia.org/wiki/File:Portrait_de_Pierre_Fauchard_par J._Le.Bel.jpg
28. *Shklar G*. John Hunter as an oral pathologist. J Hist Dent 2008; 56(1): 31−4.
29. John M. Riggs (1811−1885). Available from: https//: commons.wikimedia.org/wiki/File:Riggs_John:M.jpg

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LETTER TO THE EDITOR
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Otoscope *vs* head mirror: a comparison of commonly used diagnostic tools

Otoskop u odnosu na čeono ogledalo: poređenje često korišćenih dijagnostičkih alata

To the Editor:

The head mirror might be considered a relict of the past but it is still widely used even in developed countries [1–3]. A paper published in 1996 analyzed rural hospitals without otorhinolaryngologists in Japan and found that about 70% of the 326 analyzed hospitals had a basic otorhinolaryngology kit (aural speculum, head mirror and nasal speculum) [4]. Due to the relatively low costs, almost no necessary maintenance and the versatility the head mirror remains a very popular choice among primary practice and even otorhinolaryngologists especially in the developing countries. Our research tries to quantify the time needed for examination with the head mirror and the otoscope, the accuracy of both methods in identifying tympanic membrane lesions and colour, the user confidence in results obtained from both methods and the perceived ease of use. The purpose of this study was to find the preferred method for ear examination in the primary health care practice.

This research was done on a group of students who performed exams on a model of the external auditory canal. The model consisted of 1) cardboard box (80 mm × 60 mm × 30 mm) with a 8 mm hole in one side; 2) one polyvinyl chloride (PVC) tube (aperture diameter 27 mm) which was bent to resemble the external auditory canal and had a 5mm process on the opening that was bent towards the opening so to imitate a tragus, and the end opposite to the tragus had a perpendicular cut in it; 3) Several red and orange circular papers that had different three digit numbers on them and served as a substitute for a tympanic membrane. The size of each individual digit was 2.7 mm. One paper was put at the end of the tube (through the perpendicular cut) with the digits facing the opening with the tragus. The tube was put inside the hole of the cardboard box with the tragus side of the tube protruding from the box.

Students were given a short course on the use of the otoscope (Heine mini 3000, 4 mm speculum) and head mirror (Riester Ziegler 90 mm and 4 mm aural speculum) and everyone was allowed to try the method before the timed exam. All of the students have previously used the head mirror at least once and at most two times in their otorhinolaryngology rotations; only one of the students has previously used the otoscope.

Students were asked to examine the tube with both the head mirror and the otoscope and write down the numbers and the colour of the paper. Papers were changed between exams so no students would look at the same paper twice or influence their colleagues. Of the 51 students that completed the test 26 performed the exam with the otoscope first and 25 completed the exam with the head mirror first. For the otoscope exam, we measured the time needed for the exam from turning on to turning off the otoscope. For the head mirror exam, we measured two times: head mirror setup time and head mirror exam. Head mirror setup time was measured from the point the student took the head mirror until the student said he found a focus, and head mirror exam time was measured from the point the student took the aural speculum and the model in his hands and the end was marked when the student put down either one.

At the end of both exams the students were asked how they were confident that they correctly identified the number and paper colour with the respected method and how easy to use was that method. Confidence was graded on a 1–10 scale where 10 had the highest confidence. Ease of use was also graded on a 1–10 scale where 10 meant easiest.

All results were analyzed using the SPSS 19 software package. The difference between mean exam times for both methods and the differences between perceived confidence and ease of use were analyzed using the Mann-Whitney test. The statistical significance in misidentification frequency for both colour and numbers between the two methods was analyzed using the Fisher's exact test.

The average time needed for the otoscope exam was significantly lower than the time needed for the head mirror exam (15.033 s *vs* 32.929 s, respectively; $p < 0.05$, Mann-Whitney test) and by logic was lower than the total time needed for the head mirror setup and exam (15.033 s *vs* 61.582 s; $p < 0.001$, Mann-Whitney test) (Table 1).

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Table 1

Otoscope *vs* head mirror average times, confidence, ease of use values and significance testing

Parameter	Otoscope	Head mirror
Setup time (s), mean (95% CI)	15.03 (12.65–17.41)	27.64 (23.31–33.97) [a]
Exam time (s), mean (95% CI)		32.93 (22.88–42.79) [b]
Setup + exam time (s), mean (95% CI)		61.57 (49.17–73.97) [a]
Confidence*	8.67 (8.08–9.25)	9.14 (8.69–9.58) [c]
Ease of use*	9.14 (8.69–9.58)	7.04 (6.26–7.14) [a]

***Determined using a 0–10 scale where 10 was marked as highest confidence and easiest of use.**
[a]$p < 0.000$; [b]$p < 0.001$; [c]$p = 0.181$ (Mann Whitney test).

Totally 25.5% and 31.4% of the students misidentified numbers by using otoscope and head mirror, respectively.

The colour identifying accuracy was found to be significantly different between the two groups as the otoscope was found to be more accurate than the head mirror (7.8% vs. 33.3% misidentified, respectively; $p < 0.05$, Fisher's exact test).

Perceived confidence in identified numbers and colour was not significantly different between otoscope and head mirror exams (average grade: 8.667 *vs* 7.980, respectively; $p = 0.181$, Mann-Whitney test) but the otoscope was found to be significantly easier to use than the head mirror (average grade: 9.136 vs. 7.039, respectively; $p < 0.001$, Mann-Whitney test) (Table 1).

Ear examination is a part of everyday's practice in almost any clinical setting and especially family medicine but family physicians are not usually as skilled at ear examination as otorhinolaryngologists are. Our test group consisted of final year medical students because, we believe, they most accurately represent inexperienced and young general practitioners.

This research clearly showed that the otoscope is a lot faster tool for performing ear exams than the head mirror. The versatility of the otoscope surpasses the head mirror, as it is a lot smaller, easier to fit in a pocket and it is self-illuminating so there is no need to search for an external light source. The otoscope can also be used for: nose examination, sinus transillumination, diagnosing hereditary teleangiectasia [5–8], throat examination and eliciting pupillary reflexes. When it comes to the ease of use the students found the otoscope to be a lot easier to use [7–9].

We believe that reading the numbers from the piece of paper, as used in this study, accurately depicts identifying lesions on the tympanic membrane (e.g. perforations) and both methods showed similar accuracy. Although authors [6–9] don't agree on the colour change of the tympanic membrane as a predictive sign for *otitis media* our study showed that correct colour identifications was superior with the otoscope than with the head mirror.

It is our opinion that the otoscope is a superior tool and that every physician should keep one close at hand.

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R E F E R E N C E S

1. *Roberts-Grey G.* An Iconic Tool: Is there still a place for the head mirror? Article – EntToday. [Internet]. ENT Today 2010; [cited 2010 Dec 28]. Available from: http://www.enttoday.org/details/article/836587/An_Iconic_Tool_Is_there_still_a_place_for_tth_head_mirror.html
2. *Ibekwe TS, Ijaduola GT, Nwaorgu OG.* Tympanic membrane perforation among adults in West Africa. Otol Neurotol 2007; 28(3): 348-52.
3. *Bull T.* Color atlas of ENT diagnosis. 4th rev. ed. Stuttgart, New York: Thieme; 2003. Chapter 1, ENT Examination; p. 3–4.
4. *Ishikawa K, Tamagawa Y, Abe K, Kitamura K, Okuno M, Mizorogi N.* Otorhinolaryngological medical care by the general practitioners in rural areas. Nippon Jibiinkoka Gakkai Kaiho 1996; 99(12): 1758–63. (Japanese)
5. *Mohler ER 3rd, Doraiswamy V, Sibley A, Bernhardt BA, Pyeritz RE.* Transillumination of the fingers for vascular anomalies: a novel method for evaluating hereditary hemorrhagic telangiectasia. Genet Med 2009; 11(5): 356-8.
6. *Pichichero ME.* Acute otitis media: Part I. Improving diagnostic accuracy. Am Fam Physician 2000; 61(7): 2051-6.
7. *Richards JR, Gaylor KA, Pilgrim AJ.* Comparison of traditional otoscope to iPhone otoscope in the pediatric ED. Am J Emerg Med 2015; 33(8): 1089-92.
8. *Kuruvilla A, Shaikh N, Hoberman A, Kovačević J.* Automated diagnosis of otitis media: vocabulary and grammar. Int J Biomed Imaging 2013; 2013: 1-15.
9. *James M, Palmer O.* Instrumentation and techniques for examination of the ear, nose, throat, and sinus. Oral Maxillofac Surg Clin North Am 2012; 24(2): 167-74.

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

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Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

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

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

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

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Tabele

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

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