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MORPHOMETRIC ANALYSIS AND PREVALENCE OF PLANTAR CALCANEAL SPURS AMONG THE ADULT POPULATION OF THE PROVINCE OF VOJVODINA

MORFOMETRIJSKA ANALIZA I UČESTALOST POJAVE TABANSKIH SPINA NA PETNIM KOSTIMA ODRASLIH LJUDI U VOJVODINI

Bojana KRSTONOŠIĆ[1], Siniša S. BABOVIĆ[1], Milan MILOVIĆ[2], Radmila PERIĆ[3], Dragan TURANJANIN[1] and Milica GLEĐA[1]

Summary

Introduction. Plantar calcaneal spur is a bony outgrowth which is most often located on the inferior aspect of the calcaneus, penetrating the surrounding soft tissues. Symptomatic and asymptomatic cases of plantar calcaneal spurs have been described and their analysis suggested that they may be a variation in regular bone development, but the exact cause is still unknown. The goal of this study was to determine the size, shape and prevalence of plantar spurs in the population of the Province of Vojvodina. **Material and Methods.** This retrospective research included a total of 272 foot X-rays. The X-rays belonged to the Clinic of Orthopedic Surgery and Traumatology, Clinical Center of Vojvodina. The images were analyzed using a publicly available software Xrayline Workstation for 32-bit. **Results.** There was no statistically significant difference in the size of calcaneal spurs between sexes, as well as between the right/left foot. In regard to their shape, irregular calcaneal spurs were more frequent (61.9%) and all of the spurs were positioned horizontally. **Conclusion.** The results of this research were in agreement with the available literature data, showing no significant difference in morphometric analysis of the plantar spurs between sexes. Future researches should include data regarding the age, body weight, and comorbidities among examinees.

Key words: Heel Spur; Calcaneus; Foot Diseases; Exostoses; Radiography; Anatomy; Morphological and Microscopic Findings; Pain

Sažetak

Uvod. Tabanska petna spina predstavlja koštani trnasti nastavak koji se najčešće nalazi na donjoj strani petne kosti i prodire u okolno meko tkivo. Opisani su simptomatski i asimptomatski slučajevi prisustva petne spine, a njihovom analizom došlo se do zaključka da ona može biti posledica varijacije uobičajenog razvoja kosti, ali stvaran uzrok njenog nastanka još uvek je nepoznat. Cilj ovog rada bio je da se utvrdi veličina, oblik petnih spina i odredi njihova prevalencija u populaciji Vojvodine. **Materijal i metode.** U ovom retrospektivnom istraživanju analizirana su ukupno 272 rendgenska snimka stopala koji pripadaju Klinici za ortopedsku hirurgiju i traumatologiju Kliničkog centra Vojvodine. Snimci stopala su analizirani u javno dostupnom programu *Xrayline Workstation* 32*bit*. **Rezultati.** Nije utvrđena statistički značajna razlika u veličini analiziranih petnih spina između polova ispitanika, kao ni između desnog i levog stopala. Prema obliku petnih spina, zastupljenije su bile nepravilne (61,9%), a sve uočene spine bile su položene horizontalno. **Zaključak.** Rezultati ovog istraživanja potvrdili su podatke dobijene u dostupnoj literaturi, bez prisutne statističke značajnosti u morfometrijskoj analizi petnih spina između polova ispitanika. Buduće istraživanje bi trebalo upotpuniti podacima o godinama starosti, telesnoj masi i komorbiditetima ispitanika.

Ključne reči: petna spina; petna kost; bolesti stopala; egzostoze; radiografija; anatomija; morfološki i mikroskopski nalazi; bol

Introduction

A thorn shaped calcification, referred to as plantar calcaneal spur (PCS), is a bony outgrowth that usually occurs on the underside of the heel bone. A number of studies that have investigated symptomatic and asymptomatic cases of PSC suggest that it may be a variation in regular bone development [1], but the exact cause is still a subject of debate.

In order to examine the causes of PCS, it is necessary to describe the anatomy of the foot. The PCS originates from the calcaneal tuberosity on the posterior surface of the calcaneus, most often in the area of the medial process, but sometimes from the lateral process or groove between processes [2–7]. The morphology of the PCSs is very variable, but generally they can be categorized into two types: simple or regular spurs (triangular in shape, with defined

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Abbreviations
PCS – plantar calcaneal spur
PF – plantar fascia

borders, broad base and a sharp point) and irregular spurs (irregular in shape, with poorly defined borders) [8, 9]. The plantar fascia (PF), which consists of fibrous connective tissue, is stretched between the medial process of calcaneal tuberosity and digits of the foot. It is important in maintaining the medial longitudinal arch of the foot, as well as in receiving and transmiting forces placed on the foot across the mid-tarsal joints. The PF could be a critical structure in the PCS formation and it has been found that its thickness is significantly greater in people with PCS than in those without it [5]. Some studies found that the PCSs are pressed inside the PF, unlike others where PCSs are not attached to the fascia [10–12]. Many studies suggest that the PSC may occur as a result of the PF inflammation, which is followed by changes in the collagen structure, vascular hyperplasia and fibroblast accumulation [5].

There is a close relation between the PCS and muscles of the foot. The PCS can be a result of degenerative changes of the intrinsic group of foot muscles. Regardless to its origin, it can make a pressure onto the muscles and cause pain [2, 4, 6]. The flexor digitorum brevis muscle attaches to the medial process tuber calcanei or to the top of the PCS, if present [12]. The abductor digiti minimi can also be inserted directly into the periosteum of the PCS [11].

Close to the medial surface of the calcaneus, the posterior tibial artery bifurcates into the medial and the lateral plantar artery. These blood vessels, accompanied by their companion veins and nerves, make an intimate relationship with the PSC, if present. In such cases, pain, sense of burning and rarely paresthesia, or a disturbance of the circulation may occur [13–17]. Moreover, cause and effect relationship between the PSC and the tarsal tunnel syndrome has been described [6, 13, 14].

Many studies have confirmed the association between the heel pain and the presence of PSC, and also found the increased incidence of PSC in the elderly and obese people. In regard to the impaired quality of life of people with PCS and its great incidence in some populations, the aim of the study was to determine the incidence of the PCS and to describe its morphology in the population of the Province of Vojvodina.

Material and Methods

The study included a total of 272 X-rays of patients treated at the Clinic of Orthopedic Surgery and Traumatology of the Clinical Center of Vojvodina in a five-month-period (from July 1, 2017 to December 1, 2017). The retrospective study was performed in compliance with the principles of the Declaration of Helsinki (1964), with the approval of the Ethics Committee of the Clinical Center of Vojvodina and the Ethics Committee of the Faculty of Medicine, University of Novi Sad. Out of a total sample of 272 lateral foot X-rays, 36 showed a calcaneal spur **(Figure 1A)**. Further, they were analyzed by morphometry. The X-ray images were loaded into specialized public software for image reconstruction and analysis Xrayline Workstation for 32-bit (http://www.xrayline.com/downloads.shtml). The calcaneal spur length was measured using the Ruler Tool. As the spurs are triangular in shape, their length was measured as the distance between the midpoint of the base and the top of the triangle **(Figure 1B)**. Each spur was measured three times and the analyzed length represented the arithmetic mean of the obtained value. According to the literature data, the calcaneal spur is a thorny extension equal to or greater than 0.25 cm. Results were statistically analyzed using the Student's t-test and presented in relation to gender and orientation - right/left foot. The second part of the research was a descriptive analysis of the calcaneal spur shape. Based on the appearance and sharpness of the edges, the spurs are classified into two groups: simple (regular) and irregular. Simple spurs were considered to be triangular in shape with clear-

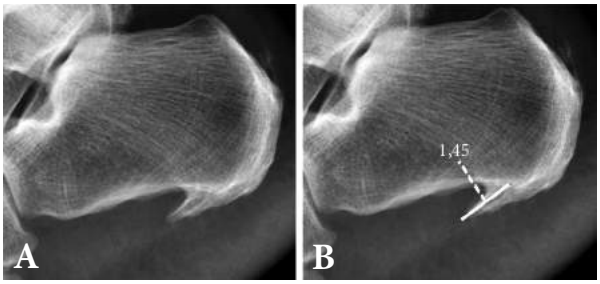

Figure 1. X-ray images: A – plantar calcaneal spur; B – measurement of plantar calcaneal spur using the Xrayline Workstation software
Slika 1. *Rendgenski snimci: A – snimak na kojem se vidi tabanska petna spina; B – snimak na kojem je pokazano kako je tabanska petna spina merena korišćenjem programa Xrayline Workstation*

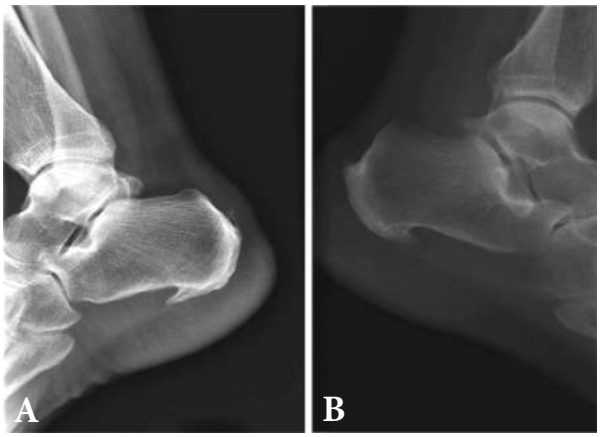

Figure 2. X-ray images: A – a regular plantar calcaneal spur; B – an irregular plantar calcaneal spur
Slika 2. *Rendgenski snimci: A – snimak na kojem se vidi pravilna tabanska petna spina; B – snimak na kojem se vidi nepravilna tabanska petna spina*

ly visible edges **(Figure 2A)**, while irregular spurs had irregular shapes and vague edges **(Figure 2B)**. Also, the direction of spur formation, i.e. the position of its tip was observed and described.

Results

Of the 272 analyzed X-rays, 36 (13.2%) showed the presence of PCS, 14 (38.9%) in male and 22 (61.1%) in female subjects. The **Table 1** shows statistically processed results of spur length, with minimum and maximum values, mean values and standard deviations. The results were presented in relation to sex distribution of subjects. We can see that there was no statistically significant difference in the spur length between male and female subjects (p = 0.43). The **Table 2** shows the spur length in males, with minimum and maximum values, mean values, and standard deviations. The results were distributed according to the presence of spur on the right/left foot. There was no statistically significant difference in spur length between the right and left foot (p = 0.2). The **Table 3** shows the statistically processed results of spur length in females, with minimum and maximum values, mean values and standard deviations. The results were distributed according to the presence of spur on the right/left foot. The **Table 3** shows no statistically significant difference in spur length between subjects' right and left foot (p = 0.27).

The second part of the study focused on the appearance of the thorny heel spurs which were classified into two groups: simple (regular) and irregular heel spurs. Accordingly, the results showed that 14 spurs (38.9%) were simple and 22 (61.1%) irregular in shape. More frequent occurrence of irregular spurs was seen in female subjects, as opposed to simple spurs, which were more frequent in males.

In regard to their direction, the PCSs were classified as horizontal or vertical. In this study, all the observed spurs had the same horizontal position, but the angle at which they were separated from the calcaneus differed **(Figure 3).** More specifically, 15 PSCs (41.7%) had a classic horizontal position, with the tips pointing forward, 16 of them (44.4%) with the tip pointing upwards, while 5 PSC (13.9%) were pointing downwards.

Table 1. Results of the morphometric analysis of plantar calcaneal spurs with sex distribution
Tabela 1. *Prikaz rezultata morfometrijske analize tabanskih petnih spina u odnosu na pol ispitanika*

Parameter *Parametar*	Male/*Muškarci*			Female/*Žene*			p
	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	
Spur length/*Dužina spine (cm)*	0.31	1.14	0.69±0.28	0.26	1.86	0.71±0.44	0.43

Table 2. Results of the morphometric analysis of plantar calcaneal spurs regarding the right and left foot of male subjects
Tabela 2. *Prikaz rezultata morfometrijske analize tabanskih petnih spina muškaraca u odnosu na desno/levo stopalo*

Parameter *Parametar*	Right foot/*Desno stopalo*			Left foot/*Levo stopalo*			p
	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	
Spur length/*Dužina spine (cm)*	0.31	1.14	0.61±0.29	0.51	1.06	0.75±0.28	0.2

Table 3. Results of the morphometric analysis of plantar calcaneal spurs regarding the right and left foot of female subjects
Tabela 3. *Prikaz rezultata morfometrijske analize tabanskih petnih spina žena u odnosu na desno/levo stopalo*

Parameter *Parametar*	Right foot/*Desno stopalo*			Left foot/*Levo stopalo*			p
	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	Minimum *Minimum*	Maximum *Maksimum*	$\overline{X}$ ± SD	
Spur length/*Dužina spine (cm)*	0.43	1.45	0.79±0.37	0.26	1.86	0.67±0.48	0.27

Discussion

Since 1900, when Plettner first used the term plantar calcaneal spur, till today, the exact etiology of spur is not known [16]. There are two hypotheses about its occurrence: the hypothesis of longitudinal traction (friction), according to which persistent friction of the PF on the surface of the calcaneus leads to the formation of a spur, and the hypothesis of vertical compression, according to which constant compression leads to microfractures of the calcaneus which consequently form connective-cartilage tumefactions. The prevailing view is that vertical compressions are more likely responsible for spur formation [16].

The goal of this study was to evaluate the prevalence of the PCSs in adults who underwent foot X-ray

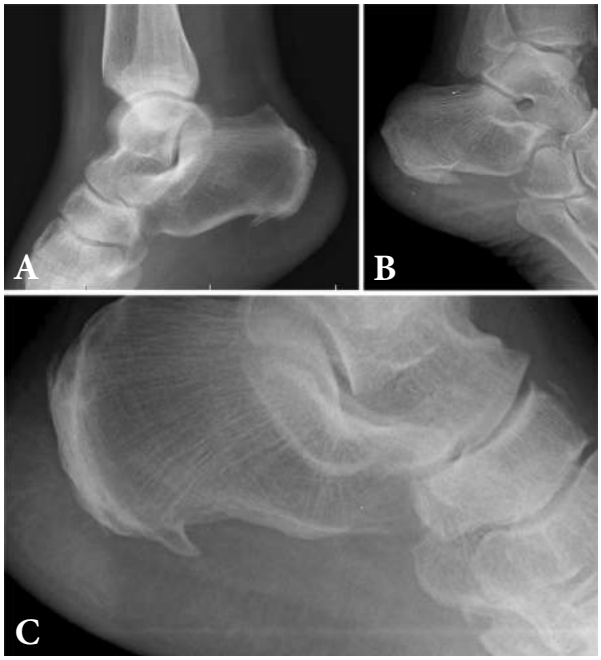

Figure 3. X-ray images that show horizontal plantar calcaneal spurs pointing in different directions: A – forward; B – upward; C – downward
Slika 3. *Rendgenski snimci na kojima se vidi horizontalno postavljena tabanska petna spina čiji je vrh različito usmeren: A – prema napred; B – prema gore; C – prema dole*

due to trauma, inflammation processes or ankle joint pain. It was found that 13.2% of the sample had PCS, which correlates with the literature data found in young to middle aged populations [18, 19–23]. Menz et al. [7] showed that 55% of their sample had PCS, with significant incidence in obese examinees, respondents who had osteoarthritis in at least one body region, or those with current or previous heel pain. That was similar to results of Bassiouni [18] who found 72% of PCS in rheumatology patients, or results of Banadda et al. [21] with 50% prevalence of PCS in hospital patients over 51 years of age.

Several theories have shown that the predisposition for spur formation depends on the sex, age, body weight, genetic predisposition, comorbidities, as well as foot position and the type of footwear [2–4, 6, 7, 15–17, 24–26]. In the young and middle aged population, the PCS prevalence was 11–21% (11% in India, 13% in Ireland, 15% in Zimbabwe, 16% in Thailand, 17% in Europe, and 21% in America) [27, 28]. The prevalence increases with age to 55% in adults over 62 years of age and up to 81% in population with osteoarthritis [12, 16]. Diseases such as diabetes mellitus and osteoporosis can be accompanied by more common formation of bony outgrowths [13, 18, 29]. Feet injuries due to inadequate jogging, prolonged running on hard surfaces, feet in pronation and intense physical activity can contribute to the formation of the PSC [2, 4–6, 13, 17, 18, 25, 29]. However, despite numerous studies the exact cause of spur formation cannot be confirmed with certainty. Between 11% and 27% of population with the PCS live without any symptoms and disorders [2, 5–7, 13, 14, 16, 17, 25, 30]. Due to this very reason, some authors consider spur to be of little importance in describing the painful heel syndrome [5] and spurs are often discovered by accident, but if present, they undoubtedly impair the quality of life.

Conclusion

The results of this study confirmed the literature data, with no statistically significant difference between sexes and orientation - right/left foot. The limitations of the study are the lack of data on age and body weight, as well as potential comorbidities of the respondents. With these data, the scientific research would be more complete and obtained results more relevant and applicable. These limitations impose the need to continue the research, expand the sample and also monitor changes in the size and shape of plantar calcaneal spurs in the analyzed sample.

References

1. Çarli AB, Tekin L, Akarsu S, Kiralp MZ. Calcaneal spur in an 18-month-old boy. Scand J Rheumatol. 2013;42(1):83-4.

2. Zhou B, Zhou Y, Tao X, Yuan C, Tang K. Classification of calcaneal spurs and their relationship with plantar fasciitis. J Foot Ankle Surg. 2015;54(4):594-600.

3. Thomas JL, Christensen JC, Kravitz SR, Mendicino RW, Schuberth JM, Vanore JV, et al. The diagnosis and treatment of heel pain: a clinical practice guideline – revision 2010. J Foot Ankle Surg. 2010;49(3 Suppl):S1-19.

4. Kosmahl EM, Kosmahl HE. Painful plantar heel, plantar fasciitis, and calcaneal spur: etiology and treatmen. J Orthop Sports Phys Ther. 1987;9(1):17-24.

5. Abreu MR, Chung CB, Mendes L, Mohana-Borges A, Trudell D, Resnick D. Plantar calcaneal enthesophytes: new observations regarding sites of origin based on radiographic, MR imaging, anatomic, and paleopathologic analysis. Skeletal Radiol. 2003;32(1):13-21.

6. Nuhmani S. Plantar fasciitis: a review of current concepts. Indian Journal of Basic and Applied Medical Research. 2012;5(2):414-8.

7. Kirkpatrick J, Yassaie O, Mirjalili SA. The plantar calcaneal spur: a review of anatomy, histology, etiology and key associations. J Anat. 2017;230(6):743-51.

8. Rubin G, Witten M. Plantar calcaneal spurs. Am J Orthop. 1963;5:38-41.

9. McCarthy DJ, Gorecki GE. The anatomical basis of inferior calcaneal lesions. A criomicrotomy study. J Am Podiatry Assoc. 1979;69(9):527-36.

10. Kumai T, Benjamin M. Heel spur formation and the subcalcaneal entesis of the plantar fascia. J Rheumatol. 2002; 29(9):1957-64.

11. Smith S, Tinley P, Gilheany M, Grills B, Kingsford A. The inferior calcaneal spur – anatomical and histological considerations. Foot. 2007;17(1):25-31.

12. Li J, Muehleman C. Anatomic relationship of heel spur to surrounding soft tissues: greater variaility than previously reported. Clin Anat. 2007;20(8):950-5.

13. Aldridge T. Diagnosing heel pain in adults. Am Fam Physician. 2004;70(2):332-8.

14. Alvarez-Nemegyei J, Canoso JJ. Heel pain: diagnosis and treatment step by step. Cleve Clin J Med. 2006;73(5):465-71.

15. Wearing SC, Smeathers JE, Urry SR, Hennig EM, Hills AP. The pathomechanics of plantar fasciitis. Sports Med. 2006;36(7):585-611.

16. Menz HB, Zammit GV, Landorf KB, Munteanu SE. Plantar calcaneal spurs in older people: longitudinal traction or vertical compression? J Foot Ankle Res. 2008;1(1):1-7.

17. Johal KS, Milner SA. Plantar fasciitis and the calcaneal spur: fact or fiction? Foot Ankle Surg. 2012;18(1):39-41.

18. Bassiouni M. Incidence of calcaneal spurs in osteo-arthrosis and rheumatoid arthritis, and in control patients. Ann Rheum Dis. 1965;24(5):490-3.

19. Alshami AM, Souvlis T, Coppieters MW. A review of plantar heel pain of neural origin: differential diagnosis and management. Man Ther. 2008;13(2):103-11.

20. Barrett SL, Day SV, Pignetti TT, Egly BR. Endoscopis heel anatomy: analysis of 200 fresh frozen specimens. J Foot Ankle Surg.1995;34(1):51-6.

21. Banadda BM, Gona O, Vaz R, Ndlovu DM. Calcaneal spurs in a black African population. Foot Ankle. 1992;13(6):352-4.

22. Benjamin M, Toumi H, Ralphs JR, Bydder G, Best TM, Milz S. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. J Anat. 2006;208(4):471-90.

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23. Berkowitz JF, Kier R, Rudicel S. Plantar fasciitis: MR imaging. Radiology. 1991;179(3):665-7.

24. Thomas JL, Christensen JC, Kravitz SR, Mendicino RW, Schuberth JM, Vanore JV, et al. The diagnosis and treatment of heel pain. J Foot Ankle Surg. 2001;40(5):329-40.

25. Aydogdu A, Akbulut H, Ege T, Tasci I, Ertugrul D, Aydogan U, et al. Increased calcaneal spur frequency in patients with obesity and type-2 diabetes mellitus. Turk J Phys Med Rehabil. 2014;60(1):12-6.

26. Malay DS. Plantar fasciitis and heel spur syndrome: a retrospective analysis. In: Vickers NS, editor. Reconstructive surgery of the foot and leg - update '96. Tucker, GA: The Podiatry Institute; 1996. p. 39-43.

27. Kullar JS, Randhawa GK, Kullar KK. A study of calcaneal enthesophytes (spurs) in Indian population. Int J Appl Basic Med Res. 2014;4(Suppl 1):S13-6.

28. Moroney PJ, O'Neill BJ, Khan-Bhambro K, O'Flanagan SJ, Keogh P, Kenny PJ. The conundrum of calcaneal spurs: do they matter? Foot Ankle Spec. 2014;7(2):95-101.

29. Peter A. Charcot's joint as a chronic complication in diabetes mellitus. Med Pregl. 2002;55(7-8):329-34.

30. Niewald M, Seegenschmiedt MH, Micke O, Graeber S, Muecke R, Schaefer V, et al. Randomized, multicenter trial on the effect of radiation therapy on plantar fasciitis (painful heel spur) comparing a standard dose with very low dose: mature results after 12 months' follow up. Int J Radiat Oncol Biol Phys. 2012;84(4):e455-62.

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LOW BACK PAIN AMONG HEALTH WORKERS

BOL U DONJEM DELU LEĐA KOD ZDRAVSTVENIH RADNIKA

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Summary

Introduction. Back pain is often present among health workers. The aim of the study was to determine the impact of gender and years of work experience on the incidence of low back pain. **Material and Methods.** A prospective cross-sectional study included 67 subjects of both genders, and it was conducted in the period between June 1 and 15, 2020, in two health centers in Serbia. The impact of gender, age, years of work experience on the incidence of low back pain was analyzed, measured by the Roland-Morris disability questionnaire. **Results.** In the examined sample (n = 67), the majority were females (55, 82.1%). The average age of the respondents was 45.5 ± 12.2 years, with an average work experience of 20.62 ± 12.03 years. Low back pain was present in 35 subjects (52.2%). There was no statistically significant difference between male and female subjects regarding the Roland-Morris disability score (3.83 ± 4.50 vs. 4.96 ± 4.53; $p > 0.05$). There was a statistically significant positive correlation between the age of subjects and the Roland-Morris disability score ($r = 0.407$; $p < 0.01$). Subjects with more years of work experience presented with a higher Roland-Morris disability score ($r = 0.371$; $p < 0.01$). **Conclusion.** Low back pain is common in older health workers with longer work experience, regardless of the level of formal education. Additional education of health workers on the application of protective attitudes, movements, and exercise, is needed in order to prevent the development of low back pain in the work environment.

Key words: Low Back Pain; Health Personnel; Quality of Life; Surveys and Questionnaires; Disability Evaluation; Risk Factors

Sažetak

Uvod. Bol u leđima je često prisutan kod zdravstvenih radnicka. Cilj istraživanja bio je utvrditi uticaj pola i godina radnog staža na bol u donjem delu leđa. **Materijal i metode.** Prospektivna studija preseka obuhvatila je 67 ispitanika, oba pola, a istraživanje je sprovedeno u periodu 1. 6. 2020−15. 6. 2020. u dva doma zdravlja u Srbiji. Analiziran je uticaj pola, starosti, godina radnog staža na prisustvo bola u donjem delu leđa, mereno Roland-Morisovim upitnikom onesposobljenosti. **Rezultati.** U ispitivanom uzorku (n = 67), većinu su činile osobe ženskog pola − 55 (82,1%). Prosečna starost ispitanika bila je 45,5 ± 12,2 godina, a ostvarili su u proseku 20,62 ± 12,03 godina radnog staža. Bol u donjem delu leđa bio je prisutan kod 35 ispitanika (52,2%). Između ispitanika muškog i ženskog pola ne postoji statistički značajna razlika u Roland-Morisovom skoru (3,83 ± 4,50 vs. 4,96 ± 4,53; $p > 0,05$). Postoji statistički značajna pozitivna povezanost starosti ispitanika i Roland-Morisovog skora ($r = 0,407$; $p < 0,01$). Ispitanici sa većim brojem godina radnog staža imaju viši Roland-Morisov skor ($r = 0,371$; $p < 0,01$). **Zaključak.** Prisustvo bola u donjem delu leđa često se registruje kod starijih zdravstvenih radnika sa dužim radnim stažom, bez obzira na nivo formalnog obrazovanja. Potrebna je dodatna edukacija zdravstvenih radnika o primeni zaštitnih stavova i pokreta, kao i vežbanja radi prevencije pojave bola u donjem delu leđa u radnom okruženju.

Ključne reči: lumbalni bol; zdravstveni radnici; kvalitet života; istraživanja i upitnici; procena onesposobljenosti; faktori rizika

Introduction

Low back pain (LBP) is defined as pain and discomfort, localized below the costal margin and above the inferior gluteal folds, with or without leg pain. Nonspecific (common) low back pain is defined as low back pain not attributable to a recognizable, known specific pathology [1–4]. Back pain is a common phenomenon with a great impact on the public health. It has been determined that more than 80% of the world's population will have at least one episode of low back pain during their lifetime [2–4]. LBP has an incidence of about 5% per year and accounts for about 3% of emergency department visits in the United States [5]. Although the current severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic has reduced healthcare care visits and treatment of patients with acute LBP (reduction by 87.2%) for several reasons [5, 6], back pain is still a growing medical and socio-economic problem [7]. It is estimated that more than a third of occupational diseases in the Nordic countries, the United States, and Japan are associated with the musculoskeletal system, and LBP has been identified as the leading cause of sick leave [8, 9]. LBP has a major impact on health workers [10], especially because it is one of the most common

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Abbreviations
LBP – low back pain
RMDQ – Roland-Morris Disability Questionnaire
SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2

musculoskeletal diseases among them [11]. It is present in all groups of health professionals [10, 12–14] in primary, secondary and tertiary healthcare services [15]. The prevalence of LBP among healthcare workers varies and in studies it ranges from 53% [12] to 92% in those involved in rotating shift work [16]. Long-term work, associated with forced body movements and postures, especially in nurses and technicians, lifting weights, with shift work, can enhance the occurrence of LBP, and thus negatively affect the work process, but also the quality of life of health workers [16–21].

The aim of the study was to determine the presence of LBP and its impact on the quality of life among healthcare professionals.

Material and Methods

A prospective cross-sectional study was conducted in the period June 1 to 15, 2020. It included a total of 67 respondents of both genders, employed at the "Bač" Health Center (30 respondents) and the "Bački Petrovac" Health Center (37 respondents), Serbia. The research was voluntary and anonymous, with a signed informed consent and previously obtained consent of the Ethics Committees of both institutions. Sociodemographic data were obtained using a questionnaire specially constructed for the purpose of this research. Respondents also completed a Roland-Morris Disability Questionnaire (RMDQ) examining the impact of LBP on the quality of life [22–26]. The RMDQ is a questionnaire for assessing the degree of disability due to lumbar spine disease, and it has been applied, translated into a number of languages, in clinical practice for many years [22–25]. This questionnaire consists of 24 questions (items) where the theoretical minimum and maximum range from 0 to 24. A higher score indicates a higher degree of disability and interference with daily functioning of respondents due to back pain. The cut-off value indicating significant functional limitations measured by this questionnaire is 14 [22–26]. The questionnaire's reliability was measured through internal consistency and it was necessary to calculate the Cronbach alpha coefficient. Acceptable values of the Cronbach alpha coefficient are those above 0.70. The Cronbach alpha coefficient per item ranges between 0.857 and 0.877, while the reliability of the entire instrument is 0.868. The questionnaire, designed for the needs of this research, contained the following variables: gender, age, years of work experience, level of formal education, presence of LBP, and length of its duration in weeks.

Data analysis, descriptive statistics such as frequencies, percentages, sample mean (arithmetic mean) and standard deviation were used. The probability level was set at $p < 0.05$. To test the differences between the parameters, Student's t test and one-factor analysis of variance (ANOVA) were used. The relationship between the two values was tested by Pearson's correlation coefficient. Statistical processing and analysis was done using the Statistical Package for the Social Sciences (SPSS) program for Windows, v. 24.

Table 1. Demographic characteristics of the sample
Tabela 1. *Demografske karakteristike uzorka*

	Total/*Ukupno* (n = 67)
Gender/*Pol*, n (%)	
Male/*Muški*	12 (17.9%)
Female/*Ženski*	55 (82.1%)
Age (years)/*Starost (godine)*, M ± SD (Min - Max)	45.5 ± 12.2 (21–73)
Level of education/*Stručna sprema*, n (%)	
High school degree/*Srednja stručna sprema*	38 (56.7%)
Applied studies degree/*Viša škola*	6 (9.0%)
University degree/*Visoka stručna sprema*	23 (34.3%)
Work experience (years)/*Dužina radnog iskustva (godine),* M ± SD (Min - Max)	20.62 ± 12.03 (1–41)
Current presence of back pain/*Trenutno prisustvo bola u leđima, n* (%)	
Yes/*Da*	35 (52.2%)
No/*Ne*	32 (47.8%)
Duration of back pain during the last episode *Dužina trajanja bola u leđima pri poslednjoj epizodi bola, n (%)*	42 (100%)
Up to 6 weeks/*Do 6 nedelja*	19 (45.2%)
6 to 12 weeks/*6 do 12 nedelja*	1 (2.4%)
More than 12 weeks/*Više od 12 nedelja*	22 (52.5%)

Legend/*Legenda:* n - number of subjects/*broj ispitanika*; M ± SD (Min - Max) = mean ± standard deviation/(minimum - maximum)/*aritmetička sredina ± standardna devijacija (minimum-maksimum)*

Results

The research included 67 respondents, 12 (17.9%) men and 55 (82.1%) women. The age of the respondents ranged from 21 to 73 years, with an average of 45.5 ± 12.2 years. Respondents had an average of 20.62 ± 12.03 years of work experience. Thirty-eight (56.7%) respondents had a high school degree, 6 (9%) had a degree in applied studies, while 23 (34.3%) respondents had a university degree as the highest level of formal education. At the moment of examination, 35 (52.2%) subjects had a back pain. Subjects provided data on the duration of LBP during the last episode. Nineteen (45.2%) of them felt pain for up to 6 weeks, in one subject (2.4%) the pain lasted from 6 to 12 weeks, while 22 (52.5%) subjects felt pain for more than 12 weeks **(Table 1).** The RMDQ scores were similar in subjects with a high school degree (5.15 ± 4.62), applied studies degree (3.33 ± 4.36) and university degree (4.17 ± 4.32; $p > 0.05$). Those subjects who currently reported back pain had a higher RMDQ score (6.31 ± 4.24) compared to those who were pain free (3.12 ± 4.23; $p < 0.01$). However, those who reported having longer pain episodes, i.e. more than 12 weeks, also had a higher average RMDQ score (7.31 ± 4.46) compared to those who had pain for less than 6 weeks (4.68 ± 3.36; $p < 0.05$) **(Table 2).**

There was a statistically significant positive correlation between the age of the subjects and the RMDQ score ($r = 0.407$; $p < 0.01$). The correlation was positive and statistically significant at the level of 0.01, i.e. older respondents had higher RMDQ scores. The years of work experience also showed a statistically significant positive correlation with the RMDQ score ($r = 0.371$; $p < 0.01$). The strength of the connection was low, but statistically significant at the level of 0.01, i.e. the longer the work experience of the respondents, the higher the RMDQ scores **(Table 3).**

There was no statistically significant difference between male and female subjects regarding the RMDQ score (3.83 ± 4.50 vs. 4.96 ± 4.53; $p > 0.05$). The average RMDQ score is 4.73 ± 4.49. The sample minimum and maximum ranged from 0 to 17.

Table 2. RMDQ score in the examined subjects
Tabela 2. *RMDQ skor kod ispitanika*

	RMDQ score/*RMDQ skor*	*p* value/*vrednost p*
Gender/*Pol*		
Male/*Muški*	3.83 ± 4.50	0.437[a]
Female/*Ženski*	4.96 ± 4.53	
*RMDQ score/*RMDQ skor*, M±SD (Min-Max)	4.73 ± 4.49 (0 – 17)	
Level of education/*Stručna sprema*		
High school degree/*Srednja stručna sprema*	5.15 ± 4.62	
Applied studies degree/*Viša škola*	3.33 ± 4.36	0.538[b]
University degree/*Visoka stručna sprema*	4.17 ± 4.32	
Current Presence of back pain/*Trenutno prisustvo bola u leđima*		
Yes/*Da*	6.31 ± 4.24	< 0.01[a]
No/*Ne*	3.12 ± 4.23	
Duration of back pain*/*Dužina trajanja bola u leđima**		
Up to 6 weeks/*Do 6 nedelja*	4.68 ± 3.36	< 0.05[a]
More than 12 weeks/*Više od 12 nedelja*	7.31 ± 4.46	

Legend/*Legenda:* RMDQ - Roland-Morris Disability Questionnaire/*RMDQ - Roland-Morisov upitnik onesposobljenosti;* M ± SD (Min - Max) = mean ± standard deviation/(minimum - maximum)/*aritmetička sredina ± standardna devijacija (minimum-maksimum)*; p - statistical significance/*statistička značajnost,* *Category 6 to 12 weeks is omitted, because only one respondent belongs to this category/*izostavljena je kategorija 6 do 12 nedelja, jer samo jedan ispitanik pripada ovoj kategoriji,* [a]Student's t–test/*Studentov t-test*; [b]ANOVA test/*ANOVA test*

Table 3. Relationship between the RMDQ score and the age of respondents and years of work experience
Tabela 3. *Povezanost RMDQ skora sa starošću ispitanika i dužinom radnog staža*

		Age (years)/*Starost (godine)*	Work experience (years)/*Radno iskustvo (godine)*
RMDQ score/*RMDQ skor*	r	0.407**	0.371**
	p	0.001	0.003

Legend/*Legenda:* RMDQ - Roland-Morris Disability Questionnaire/*Roland-Morisov upitnik onesposobljenosti*; r- Pearson's correlation coefficient/*Pirsonov koeficijent korelacije*; p - statistical significance/*statistička značajnost*, **Correlation is significant at the 0.01 level (2-tailed)/*korelacija značajna na nivou 0.01*

Discussion

Healthcare workers are among the group with high risk of LBP [12]. Work in primary, secondary and tertiary healthcare institutions has its specificities for all profiles of healthcare workers, and it is often associated with musculoskeletal diseases [15]. Our investigation included 55 (82.1%) female participants, which is in line with other studies, where certain profiles of health workers, especially nurses, are dominated by women [12, 27]. The age of the sample in our study ranged from 21 to 73 years, with an average of 45.5 ± 12.2 years, which is significantly higher compared to other studies [11, 27]. Age, body mass index, and female gender were the most commonly reported individual risk factors for LBP [10]. In our study, respondents had an average of 20.62 ± 12.03 years of work experience. A number of studies indicate an association between years of work and LBP [13, 16]. A study including 120 health workers, by Zahra NAI et al, found that longer working hours may be associated with higher LBP prevalence [27]. The study including 87 respondents by Peković D, showed that mild pain dominates in the group of respondents with up to 10 years of work experience, examinees with 11 to 30 years of work experience mostly present with moderate pain, while severe/strong pain is dominant in the group of respondents with over 30 years of work experience. In the mentioned research, out of the total number of respondents who experienced lumbar pain, 63% reported that lifting loads was the main cause of pain, 17% reported bending over, and 7% stated that twisting the torso was the main cause of pain [16]. In our research, 38 (56.7%) respondents had a high school degree, 6 (9%) had applied studies degree, while 23 (34.3%) respondents had a university degree as the highest level of formal education. The level of education and qualifications are profiling the position and type of job in the health system. Zahra NAI et al, confirmed a highly significant relationship between LBP and age, sex, education, qualifications and years of experience [27]. In our study, we analyzed LBP in healthcare workers in primary healthcare. Several studies have found that health workers, especially those employed in secondary and tertiary healthcare facilities, are at increased risk for developing LBP [13, 15]. In the research by Peković D, 87% of respondents (nurses/technicians employed in a tertiary healthcare institution) stated that they had LBP in the last year [16]. Nurses and physical therapists were more susceptible to LBP [10], but it is also present among non-medical workers employed in the health sector [27]. In a study by Şimşek Ş. et al, including 1,682 healthcare workers, LBP was most common among medical secretaries (56.9%), and factors that increased the risk for LBP were advanced age, female gender, high body mass index, married status, lack of regular exercise, working for more than 4 hours while standing or sitting at the desk, using a computer for more than 4 hours, higher number of years of service, and low job satisfaction [12]. In our study, LBP was present in 35 (52.2%) subjects, which is consistent with other studies [12, 26]. In relation to the duration of LBP, 19 subjects (45.2%) felt pain for up to 6 weeks, in one subject (2.4%) these sensations lasted from 6 to 12 weeks, while 22 (52.5%) subjects felt pain for more than 12 weeks, which indicates that it is a significant problem among healthcare workers in primary healthcare. In a study including 765 subjects, Yokota J et al, found that the overall prevalence of LBP was 64.6% (acute LBP in 47.5%, chronic LBP in 17.1%) [28]. The quality of life of people with LBP was influenced by a number of factors, of which age, level of education, psychosocial factors, job satisfaction, as well as work-related factors were very important [29–31]. Changes in quality of life in people with LBP over time can be easily objectified by applying RMDQ [31]. In our study, no statistically significant difference was found between male and female subjects regarding the RMDQ score, or between subjects with different levels of education. People with higher education and with more work experience are more aware of preventive measures for chronic conditions [28–31]. The average RMDQ score in our examinees was 4.73 ± 4.49. Subjects who had LBP for more than 12 weeks had a higher average RMDQ score compared to those with LBP shorter than 6 weeks (7.31 ± 4.46 vs. 4.68 ± 3.36; $p < 0.05$). The presence of chronic back pain leads to a higher degree of disability and a lower quality of life. In a study by Li L et al, on the presence of LBP measured by the Roland-Morris questionnaire, 370 nurses (70.7%) scored 1 to 2 points, 128 (24.5%) scored 3 to 4 points, 20 (3.8%) scored 5 to 6 points, and five nurses (1.0%) scored 7 points [31]. In our study, older respondents with longer work experience had a higher degree of disability, as measured by RMDQ, indicating a lower quality of life due to LBP. The presence of chronic LBP can affect day-to-day functioning in the work environment and can influence healthcare workers to change their careers. A study by Li L et al, found that more than half of the orthopedic nurses (51.1%) planned to quit, and 5.8% thought of leaving their job due to LBP [31]. The application of preventive programs and exercises among healthcare workers can reduce the incidence of musculoskeletal diseases, including LBP. Exercise once a week for 6 months combined with five sessions of back care counseling after working hours in real-life settings effectively reduced the intensity of LBP, work interference due to LBP, and fear of pain [19, 32].

Conclusion

The results of our study showed that the presence of low back pain is common in older health workers with longer work experience, regardless of the level of formal education. This study has some limitations, such as small number of respondents, lack of data on regular physical activity and exercise, or additional jobs, hobbies and activities out-

side the workplace, which may affect the development of low back pain. Additional education of health workers regarding protective attitudes, movements, and exercise, is needed in order to prevent the occurrence of low back pain in the work environment and improve the quality of life of health workers.

References

1. Burton AK, Balague F, Cardon G, Eriksen HR, Henrotin Y, Lahad A, et al. Chapter 2. European guidelines for prevention in low back pain: November 2004. Eur Spine J. 2006;15(Suppl 2):S136-68.

2. Dagenais S, Caro J, Haldeman S. A systematic review of low back pain cost of illness studies in the United States and internationally. Spine J. 2008;8(1):8-20.

3. Vidal-Conti J. Relationship between low back pain and sport practice in young people. International Journal of Orthopedics and Rehabilitation. 2014;1:67-73.

4. Rubin DI. Epidemiology and risk factors for spine pain. Neurol Clin. 2007;25(2):353-71.

5. Della-Giustina D. Evaluation and treatment of acute back pain in the emergency department. Emerg Med Clin North Am. 2015;33(2):311-26.

6. Borsa S, Pluderi M, Carrabba G, Ampollini A, Pirovano M, Lombardi F, et al. Impact of COVID-19 outbreak on acute low back pain. World Neurosurg. 2020;139:749.

7. Kent PM, Keating JL. The epidemiology of low back pain in primary care. Chiropr Osteopat. 2005;13:13.

8. Opsahl J, Eriksen HR, Tveito TH. Do expectancies of return to work and job satisfaction predict actual return to work in workers with long lasting LBP? BMC Musculoskelet Disord. 2016;17(1):481.

9. Hoy D, March L, Brooks P, Blyth F, Woolf A, Bain C, et al. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73(6):968-74.

10. Al Amer HS. Low back pain prevalence and risk factors among health workers in Saudi Arabia: a systematic review and meta-analysis. J Occup Health. 2020;62(1):e12155.

11. Almalki M, Alkhudhayri MH, Batarfi AA, Alrumaihi SK, Alshehri SH, Aleissa SI, et al. Prevalence of low back pain among medical practitioners in a tertiary care hospital in Riyadh. Saudi Journal of Sports Medicine. 2016;16(3):205-9.

12. Şimşek Ş, Yağcı N, Şenol H. Prevalence and risk factors of low back pain among healthcare workers in Denizli. Agri. 2017;29(2):71-8.

13. Skela-Savič B, Pesjak K, Hvalič-Touzery S. Low back pain among nurses in Slovenian hospitals: cross-sectional study. Int Nurs Rev. 2017;64(4):544-51.

14. Citko A, Górski S, Marcinowicz L, Górska A. Sedentary lifestyle and nonspecific low back pain in medical personnel in North-East Poland. Biomed Res Int. 2018;2018:1965807.

15. Alnaami I, Awadalla NJ, Alkhairy M, Alburidy S, Alqarni A, Algarni A, et al. Prevalence and factors associated with low back pain among health care workers in southwestern Saudi Arabia. BMC Musculoskelet Disord. 2019;20(1):56.

16. Pekovic D. Study of risk factor resulting in occurrence of lumbar syndrome in nurses. Sestrinska reč. 2018;21(76):37-40.

17. Beyer F, Geier F, Bredow J, Oppermann J, Schmidt A, Eysel P, et al. Non-operative treatment of lumbar spinal stenosis. Technol Health Care. 2016;24(4):551-7.

18. Beyer F, Geier F, Bredow J, Oppermann J, Eysel P, Sobottke R. Influence of spinopelvic parameters on non-operative treatment of lumbal spinal stenosis. Technol Health Care. 2015;23(6):871-9.

19. Soler-Font M, Ramada JM, van Zon SKR, Almansa J, Bültmann U, Serra C. Multifaceted intervention for the prevention and management of musculoskeletal pain in nursing staff: results of a cluster randomized controlled trial. PLoS One. 2019;14(11):e0225198.

20. Al-Mutairi MD. Quality of life among nurses with low back pain: a review. Open J Nurs. 2019;9(11):1138-42.

21. Roland M, Morris R. A study of the natural history of back pain. Part 1: development of a reliable and sensitive measure of disability in low back pain. Spine. 1983;8(2):141-4.

22. Roland MO. A critical review of the evidence for pain-spasm-pain cycle in spinal disorders. Clin Biomech. 1986;1(2):102-9.

23. Nusbaum L, Natour J, Ferraz MB, Goldenberg J. Translation, adaptation and validation of the Roland-Morris questionnaire - Brazil Roland-Morris. Braz J Med Biol Res. 2001;34(2):203-10.

24. Roland M, Fairbank J. The Roland-Morris disability questionnaire and the Oswestry Disability Questionnaire. Spine. 2000;25(24):3115-24.

25. The Roland-Morris Disability Questionnaire [Internet]. [cited 2020 Aug 21]. Available from: http://www.rmdq.org/.

26. Žilić I, Tudor A, Ružić L. Povezanost razine tjelesne aktivnosti i prevalencije križobolje kod djelatnika bolnice Lipik. Hrvatski športskomedicinski vjesnik. 2017;32(1-2):59-66.

27. Zahra NAI, Elmoaty Sheha EAA, Elsayed HA. Low back pain, disability and quality of life among health care workers. International Journal of Pharmaceutical Research and Allied Sciences. 2020;9(2):34-44.

28. Yokota J, Fukutani N, Nin K, Yamanaka H, Yasuda M, Tashiro Y, et al. Association of low back pain with presenteeism in hospital nursing staff. J Occup Health. 2019;61(3):219-26.

29. Patrick N, Emanski E, Knaub M. Acute and chronic low back pain. Med Clin North Am. 2014;98(4):777-89.

30. Gurcay E, Bal A, Eksioglu E, Hasturk AE, Gurcay AG, Cakci A. Acute low back pain: clinical course and prognostic factors. Disabil Rehabil. 2009;31(10):840-5.

31. Li L, Deng X, Zhang H, Yang H, Chen J, Hou X, et al. A cross-sectional survey of low back pain in nurses working in orthopedic departments. Workplace Health Saf. 2019;67(5):218-30.

32. Suni JH, Kolu P, Tokola K, Raitanen J, Rinne M, Taulaniemi A, et al. Effectiveness and cost-effectiveness of neuromuscular exercise and back care counseling in female healthcare workers with recurrent non-specific low back pain: a blinded four-arm randomized controlled trial. BMC Public Health. 2018;18(1):1376.

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THE ROLE OF LYMPHOSCINTIGRAPHY IN THE DIAGNOSIS OF LYMPHEDEMA

ULOGA LIMFOSCINTIGRAFIJE U DIJAGNOSTICI LIMFEDEMA

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Summary

Introduction. Lymphedema is a chronic disease of the lymphatic system that often remains undiagnosed or poorly diagnosed and can lead to severe and disabling swelling of the extremities. The aim of this paper was to review the literature on lymphoscintigraphy as a nuclear medicine imaging technique in the diagnosis of lymphedema, as well as to present clinical cases where lymphoscintigraphy was performed due to edema of unknown origin. **Material and Methods.** A literature review was performed using PubMed and manual search. Additionally, characteristics of diagnostic radiopharmaceuticals, methodological aspects of lymphoscintigraphy and interpretation criteria used in our department were presented in two clinical cases. **Results.** Literature data analysis showed that in the diagnosis of lymphedema, lymphoscintigraphy is a reliable diagnostic method in evaluation of the functional capacity of the lymphatic system, with a sensitivity of up to 96% and a specificity of 100%. In the presented clinical cases, lymphoscintigraphy diagnosed functional dysfunction/obstruction of the lymphatic pathways in the lower extremities. **Conclusion.** Lymphoscintigraphy is a safe and reliable method in the diagnostic algorithm of patients with lymphedema, also valuable in monitoring the condition after the applied therapeutic modalities.

Key words: Lymphedema; Lymphoscintigraphy; Diagnostic Imaging; Risk Factors; Lymphatic Vessels; Edema

Sažetak

Uvod. Limfedem je hronična bolest limfnog sistema koja često ostaje nedijagnostifikovana ili nedovoljno dijagnostifikovana i može dovesti do ozbiljnog i onesposobljavajućeg otoka ekstremiteta. Cilj ovog rada bio je pregled literature o limfoscintigrafiji kao nuklearno medicinskoj imidžing metodi u dijagnostici limfedema kao i prikaz kliničkih slučajeva gde je limfoscintigrafija urađena zbog edema nepoznatog porekla. **Materijal i metode.** Izvršen je pregled literature korišćenjem PubMed-a i drugih baza podataka. Pored toga, karakteristike radioobeleživa ča, metodološki aspekti limfoscintigrafije i kriterijumi za interpretaciju na našem odeljenju predstavljeni su kroz dva prikaza slučaja. **Rezultati.** Podaci iz literature potvrđuju limfoscintigrafiju kao pouzdanu dijagnostičku metodu u proceni funkcionalne sposobnosti limfnog sistema, sa senzitivnošću do 96% i specifičnošću od 100% u dijagnozi limfedema. U prikazanim kliničkim slučajevima limfoscintigrafijom je dijagnostifikovana funkcionalna disfunkcija/opstrukcija limfnih puteva u donjim ekstremitetima. **Zaključak.** Limfoscintigrafija je sigurna i pouzdana metoda u dijagnostičkom algoritmu pacijenata sa limfedemom, takođe je dragocena u praćenju stanja nakon primenjenih terapijskih modaliteta.

Ključne reči: limfedem; limfoscintigrafija; dijagnostički imidžing; faktori rizika; limfni sudovi; edem

Introduction

Lymphedema is a chronic disease characterized by reduced lymph transport, usually with swelling of one or more extremities and sporadically of the trunk and genitals [1]. Lymphedema is a chronic disease of the lymphatic system that often remains undiagnosed or poorly diagnosed potentially leading to severe and disabling swelling of the extremities. Fluid accumulates in the interstitial space as a result of the imbalance between the formation and reabsorption of lymph. Due to the insufficiency of the lymphatic system, there is an increase in osmotic pressure in the tissue and the consequent accumulation of fluid, which leads to swelling. Although the disease is not associated with pain, it can have a great impact on the quality of life of patients [2].

Swelling is associated with a feeling of heaviness, discomfort and reduced mobility of the extremities and they are initially pitting, but due to the longer duration of the disease there is an inflammatory and immune response of the body which is characterized by tissue infiltration with mononuclear cells, fibroblasts and adipocytes, which eventually leads to fibrosis of the skin and subcutaneous tissue and formation of hard edema [1]. If the treatment of the disease is not started on time it progresses and affects the skin, which becomes hyperkera-

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Abbreviations

99mTc-SbSC	– Technetium-99m-antimony sulfide colloid
99mTc-SC	– Technetium-99mTc-sulfur colloid
99mTc-HSA	– Technetium-99mTc-human serum albumin
LEHR	– low-energy high-resolution collimator
MBq	– megabecquerels
AP	– anterior-posterior

totic, hyperpigmented, papillomatous or verrucous with increased turgor. In the end, the skin is at risk of developing ulcerations and infections, which additionally affects the quality of life. Lymphedema may be primary or secondary.

The prevalence of primary edema is approximately 1.15 per 100,000 people under the age of 20, with a higher incidence in females [3]. Primary lymphedema may be caused by agenesis, hypoplasia, hyperplasia, or lymphatic obstruction. There are three clinical subtypes of primary lymphedema: congenital lymphedema, which occurs immediately after birth, lymphedema praecox, which occurs around puberty, and lymphedema tarda, which usually begins after the age of 35. At least 20 genes are associated with an inherited form of lymphedema [5].

Secondary lymphedema is an acquired condition that occurs because of injury or obstruction of lymph vessels that were previously normal. The most common cause of secondary lymphedema in the world is lymphatic filariasis [1]. In developed countries, the most common cause of secondary lymphedema is surgical excision or irradiation of axillary or inguinal lymph nodes in the treatment of cancers such as breast, endometrial, cervical, prostate cancer, sarcoma and melanoma. Lymphedema of the arms occurs in 14–40% of patients with breast cancer after surgery or completed radiotherapy [3, 4].

Advanced stages of lymphedema are most often diagnosed clinically, and earlier stages of the disease often require additional diagnostic procedures such as: lymphoscintigraphy, direct and indirect lymphography, magnetic resonance imaging, computed tomography and ultrasonography [1].

The aim of this paper was literature review on lymphoscintigraphy as a diagnostic method in the diagnosis of lymphedema, as well as presentation of two cases where lymphoscintigraphy was performed due to lymphedema.

Material and Methods

A literature review was performed using PubMed and manual search. Two cases were presented, as well as a lymphoscintigraphy protocol and findings with criteria for interpretation.

Results

Lymphoscintigraphy is a reliable method in diagnosing lymphedema, and due to low amounts of radioactivity, it can be relatively safely repeated several times [7–9]. In this diagnostic procedure, a radiotracer is injected into the soft tissue of the re-

Table 1. Primary and secondary lymphedema (clinical classification) (1)
Tabela 1. *Primarni i sekundarni limfedem (klinička klasifikacija) (1)*

Primary lymphedema/*Primarni limfedem*	**Secondary lymphedema/*Sekundarni limfedem***
Sporadic lymphedema (cause unknown)	Infection/*Infekcija*
Sporadični limfedem (uzrok nepoznat)	Bacterial lymphanginitis/*Bakterijski limfanginitis*
Genetic disorders/*Genetski poremećaji*	Lymphogranuloma venereum
Milroy's disease/*Milrojeva bolest*	*Limfogranuloma venereum*
Meige's disease/*Meova bolest*	Filariasis/*Filarijaza*
Cholestasis lymphedema/*Holestazni limfedem*	Tuberculosis/*Tuberkuloza*
Henakam's lymphangiectasia	Malignant lymph node infiltration
Henekamova limfangiektazija	*Maligna infiltracija limfnih čvorova*
Emberger's syndrome/*Embergerov sindrom*	Lymphoma/*Limfom*
Microcephaly-lymphedema syndrome	Prostate cancer/*Karcinom prostate*
Mikrocefalija-limfedem sindrom	Other cancers/*Drugi karcinomi*
Hypotrichosis-lymphedema-telangiectasia	Surgical or radiotherapy of axillary or inguinal lymph
Hipotrihoza-limfedem-teleangiektazija	nodes in the treatment of cancer/*Operativna ili radioterapija aksilarnih ili ingvinalnih limfnih čvorova u lečenju karcinoma*
Chromosomal aneuploidies	Iatrogenic (most often during vascular surgery or saphenous vein preparation)/*Jatrogeno (najčešće u toku vaskularnih operacija ili preparacije vene safena)*
Hromozomske aneuploidije	Diverse/*Raznovrsno*
Turner syndrome/*Tarnerov sindorm*	Contact dermatitis/*Kontaktni dermatitis*
Klinefelter's syndrome/*Klinefelterov sindrom*	Podoconiosis/*Podokonioza*
Trisomy 13,18 or 21 chromosomes	Rheumatoid arthritis/*Reumatoidni artritis*
Trizomija 13, 18 ili 21. hromozoma	Pregnancy/*Trudnoća*
Other disorders associated with primary lymphedema	
Drugi poremećaji u vezi sa primarnim limfedemom	
Noonan's syndrome/*Nunanov sindrom*	
Parker Weber syndrome/*Parker Veberov sindrom*	
Yellow nail syndrome/*Sindrom žutih noktiju*	
Intestinal lymphangiectasia syndrome	
Sindrom crevne limfangiektazije	
Neurofibromatosis type 1/*Neurofibromatoza tip 1*	

gion of interest and then the lymphatic pathways and lymph nodes are evaluated. Lymphoscintigraphy can be both quantitative and qualitative. Quantitative lymphoscintigraphy is based on the measurement of various quantitative parameters in the diagnosis of lymphedema, while qualitative lymphoscintigraphy provides insight into the morphology of the lymphatic system. Currently, there are no standardized guidelines for lymphoscintigraphy of lymphedemas. Consequently, each institution has its own protocol, adapted to available radiotracers, imaging systems and their strategic system.

Several radiotracers can be used for lymphoscintigraphy, and some of them are: Technetium-99m-antimony sulfide colloid (99mTc-SbSC), 99mTc-sulfur colloid (99mTc-SC) filtered or unfiltered, 99mTc-human serum albumin (99mTc-HSA), and 99mTc-dextran. The main difference between these radiotracers is the size of their particles. Smaller particles can enter the blood vessels and increase the background activity, and on the other hand, large particles cannot enter the lymphatic system at all [10]. It is believed that the best size of the particles is between 50 – 70 nm [11]. The particle size of 99mTc-SC is larger than other radiotracers and this can lead to a delayed transit through the lymphatic system and to non-visualization of lymphatic pathways [12]. Smaller particles of 99mTc-SbSC and 99mTc-HAS allow faster study and better display

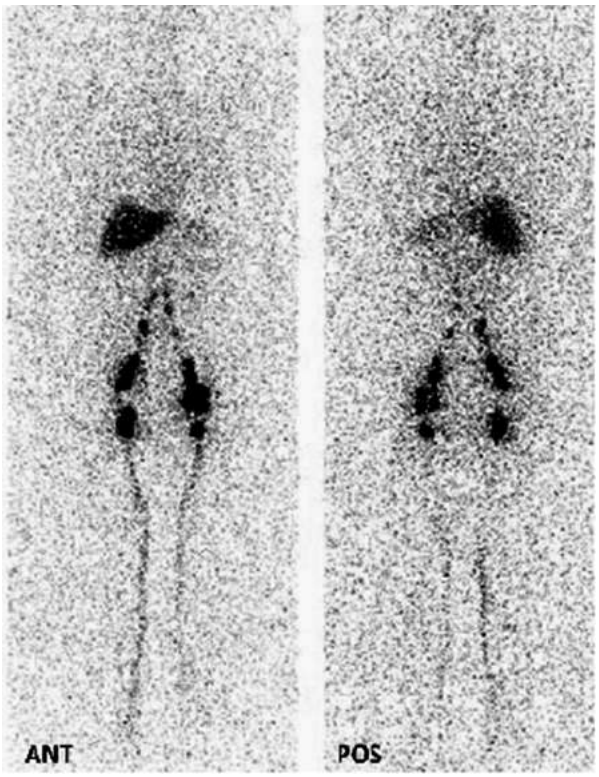

Figure 1. Normal lymphoscintigraphic finding 4h after administration of radiotracer [17]
Slika 1. *Normalan limfoscintigrafski nalaz 4 h nakon aplikacije radioobeleživa*č*a [17]*

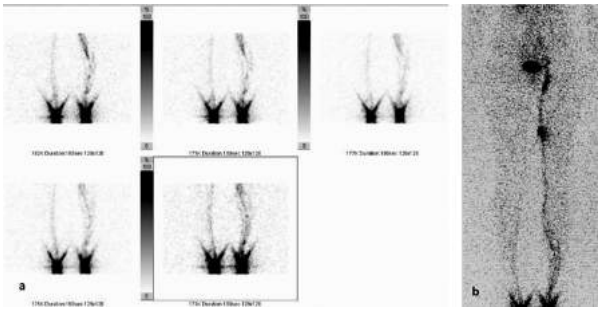

Figure 2. Anterior-posterior (AP) image, early dynamic study of lower legs (a) and early whole body image (b) showing reduced number of lymph vessels in the right lower leg and slowed flow of radiotracer in the right leg
Slika 2. *Anteriorni i posteriorni snimak, rana dinamička studija potkolenica (a) i rani snimak celog tela (b) koji pokazuju smanjen broj limfnih sudova u desnoj potkolenici i usporen protok radioobeleživa*č*a u desnoj nozi*

of lymphatic pathways [10, 13]. Radiotracer application may be subcutaneous, intradermal, or subfascial. It is debatable which method of application is the best. Some authors emphasize the need for the application of radiotracers both subcutaneously and subfascially, in order to examine both superficial and deep lymphatic pathways during the same study [14]. Also, the amount of administered activity varies from institution to institution and the type of performed study. In the vast majority of cases of limb lymphedema, the radiotracer is administered in both extremities. Exceptionally, in cases with chylous reflux, the radiotracer is administered to the healthy limb. Regarding the procedure, some authors recommend a dynamic study after the administration of the radiotracer, while others practice the whole body scanning at different time intervals from the administration of the radiotracer. It is recommended to perform recording using a low-energy high-resolution collimator (LEHR) and the whole body scan speed during acquisition is 10 cm/min. In case when early images do not show lymphatic pathways, stress activities such as walking, limb massage or pressing the ball are recommended. Changes in lymphoscintigraphy after stress activity may predict a good response to physical treatment in patients with lymphedema [15]. The sensitivity of qualitative lymphoscintigraphy in the diagnosis of lymphedema is 70%, and if quantitative parameters are included, sensitivity can go up to 100% [16]. Qualitative lymphoscintigraphy in the diagnosis of lymphedema is performed at our Department of Nuclear Medicine of the Clinical Center of Vojvodina. The 99mTc-SbSC is the most commonly used radiotracer, due to optimal characteristics (particle size) and long standing experience in preparation. The radiotracer is administered subcutaneously in the area of the dorsum of the foot (dorsum of the hand) between the roots of the first and second toe bilaterally and simultaneously. The administered amount of radioactivity is 30 – 50 megabecquerels (MBq). After the application of the radiotracer, a dynamic study is

Table 2. Stages of lymphedema (6)
Tabela 2. *Stadijumi limfedema (6)*

Stage 0 (or Ia) *Stadijum 0 (ili Ia)*	Latent or subclinical stage of the disease without swelling, despite slow lymph transport. This condition can last for months or years before the swelling occurs/*Latentni ili supklinički stadijum bolesti bez prisustva otoka uprkos usporenom transportu limfe. Ovakvo stanje može da traje mesecima ili godinama pre javljanja otoka*
Stage I *Stadijum I*	Early accumulation of fluid that is relatively full of proteins and the formation of swelling that passes during the elevation of the extremities. An increased number of proliferative cells can be observed/*Rano nakupljanje tečnosti koja je relativno puna proteina i stvaranje otoka koji prolazi prilikom elevacije ekstremiteta. Može da se uoči povećan broj proliferativnih ćelija*
Stage II *Stadijum II*	Elevation of the extremities rarely leads to a reduction in swelling, a clear presence of pitting edema, while in the late second stage pitting edema is not so pronounced due to the accumulation of fat and the formation of connective tissue/*Elevacija ekstremiteta retko dovodi do smanjenja otoka, jasno prisustvo testastog edema, dok u kasnom II stadijumu testasti edem nije toliko izražen zbog nakupljanja masti i stvaranja vezivnog tkiva*
Stage III *Stadijum III*	Lymphatic elephantiasis, the presence of trophic changes in the skin, presence of deposits of adipose tissue and connective tissue, the skin becomes papillomatous or verrucous *Limfatična elefantijaza, prisustvo trofičkih promena kože, prisustvo depozita masnog tkiva i vezivnog tkiva, koža postaje papilomatozno ili verukozno izmenjena*

performed for 30 minutes, covering the region from the application site to the inguinal regions for the lower extremities, i.e. axillary regions for the upper extremities, followed by static images of the region of thighs (upper arms) and inguinal regions (axillary regions) at intervals of 45 min, 90 min, 4h and 24h after the administration of radiotracer, as well as static imaging of the liver. We must note that the time intervals of static image acquisition time of the regions of interest vary in accordance with clinical needs, but also from patient to patient. If there is no visualization of lymphatic pathway during the early dynamic study, the patient is advised to take a short walk or to massage

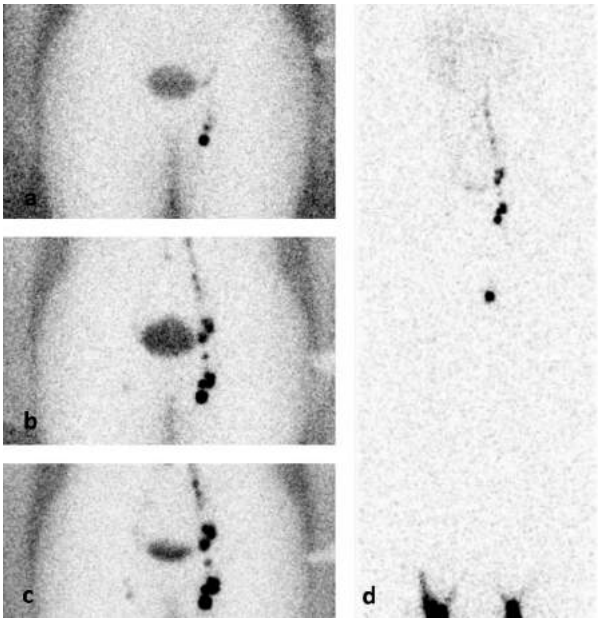

Figure 3. Static images at 45 min (a), 90 min (b), 4h (c) and a late whole body image after 24h
Slika 3. *Statički snimci nakon 45 minuta (a), 90 minuta (b), 4 h (c) i kasni snimak celo tela nakon 24 h*

the extremity. Normal lymphoscintigraphic finding represents symmetrical movement of radiotracer in the extremities, visualization of discrete lymphatic pathways; early visualization of regional lymph nodes usually within 15 – 20 minutes [14] as well as visualization of the liver within one hour **(Figure 1)** [17, 18]. Some studies suggest that visualization of popliteal lymph nodes is normal in the lower extremities [16], while other studies consider that visualization of popliteal lymph nodes is a sign of lymphatic system dysfunction [19]. Pathological finding of lymphoscintigraphy represents: asymmetric presentation of regional lymph nodes, or non-visualization of lymph nodes in severe cases of the disease, dermal backflow of radiotracer that occurs due to the existence of smaller collateral lymphatic pathways [14], interrupted or blocked flow of radiotracer, dilated or collateral lymphatic pathways as well as reduced number of regional lymph nodes.

Although some authors believe that lymphoscintigraphic findings are different in primary and secondary lymphedema [14], most studies claim that these two entities cannot be distinguished by lymphoscintigraphy [18]. Before lymphoscintigraphy, it is necessary to exclude the most common causes of extremity swelling such as renal failure, nephrotic syndrome, hypoalbuminemia, congestive heart failure, pulmonary hypertension, iatrogenic edema caused by drugs, obesity and pregnancy.

Case 1.

Figure 2 shows a lymphoscintigraphic finding indicating a reduced number of lymph vessels in the right lower leg and delayed kinetics of radiotracer in the right leg in a 35-year-old female patient referred due to stage I lymphedema of the right lower leg. Vascular etiology of the swelling was ruled out by the vascular surgeon and by color Doppler examination of lower extremities; laboratory findings and ultrasound examination of the abdomen excluded liver and kidney pathology, whereas gynecological examination excluded the possibility of

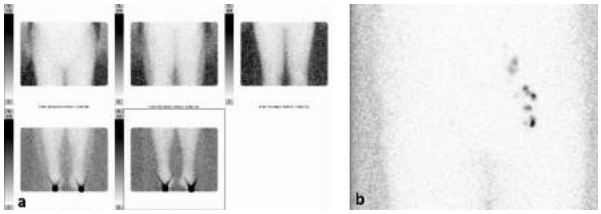

Figure 4. Early dynamic study indicating the absence of radiotracer kinetics on both sides (a) and delayed static image 3h after radiotracer administration, without showing inguinal lymph nodes on the right side (b)
Slika 4. *Rana dinamička studija koja ukazuje na odsustvo kinetike radioobeleživa*č*a obostrano (a) i odloženi statitčki snimak 3 h nakon aplikacije radioobeleživa*č*a, bez prikazivanja ingvinalnih limfnih čvorova desno (b)*

gynecological pathology and pregnancy. The patient has been an athlete for many years, and the internist's examination ruled out the possibility of heart failure and pulmonary hypertension.

Figure 3 shows delayed static images after physical activity in the same patient at 45, 90 min, and 4 h after radiotracer administration, as well as a late whole body imaging after 24 h. These images showed a slow and late visualization of lymph nodes in the right inguinum, and a reduced number of right inguinal lymph nodes. The finding on the left was timely and orderly.

Case 2.

Figure 4 shows a lymphoscintigraphic finding indicating delayed radiotracer kinetics bilaterally, more to the right in a 55-year-old male patient referred for stage II lymohedema of the right lower leg. The patient was controlled by a hematologist for 25 years due to suspected myeloproliferative syndrome, although it was not confirmed by histopathological examination of the bone marrow. Vascular cause of swelling was ruled out by color Doppler and vascular surgeon examination, laboratory analysis ruled out liver and kidney pathology, internist examination revealed cardiac pathology which was not the cause of unilateral leg swelling. The patient was using a selective beta blocker, torasemide, and a combination of antiplatelet therapy. He also had a history of right ankel fracture.

Figure 4 (b) shows a delayed static image after physical activity in the same patient. The finding on the right indicates practical absence of a radiotracer in the right inguinal region, while in the left inguinal region visualization of normal number of lymph nodes is delayed.

Discussion

Lymphoscintigraphy is considered to be one of the main diagnostic methods in the diagnosis of lymphedema and visualization of lymphatic pathways [20, 21]. However, the use of lymphoscintigraphy for diagnostic purposes varies worldwide from being used in some centers for each lymphedema, while in some centers it is rarely used. Lymphoscintigraphy is a method based on the transport role of the lymphatic system by which interstitial fluid with molecules is transported from the interstitial space to the vascular compartment. The radiotracer injected into the interstitial space is transported by lymphatic pathways and through the lymph nodes, all of which is monitored using a gamma camera that registers radioactivity. In this way, an image of the lymphatic system is obtained. The speed of movement of the radiotracer through the lymphatic system depends on the particle size of the radiotracer itself. It is best to use a radiotracer that has particle size of 50 – 70 nm [12] such as 99mTc-SbSC and 99mTc-HAS. Also, the kinetics of radiotracer is affected by physical activity, and therefore patients should be encouraged to walk or massage the extremities in case there is no visualization of lymphatic pathways after a dynamic study lasting 30 minutes. Our department of nuclear medicine performs qualitative lymphoscintigraphy, which aims to show the morphology of the lymphatic system. Scintigraphic findings from these two cases imply different degrees of lymphatic dysfunction. In our first case we observed unilateral presentation of lymphatic dysfunction. The patient had reduced number of lymphatic vessels in the right lower extremity, delayed kinetics of radiotracer with consequent delayed visualization of inguinal lymph nodes, as well as reduced number of inguinal lymph nodes. Clinical presentation was in correlation with the scintigraphic finding. On the contrary, bilateral presentation of lymphatic dysfunction and lack of correlation between scintigraphy and clinical sings were observed in the second case. This patient showed absence of kinetic radiotracer in the right lower extremity and delayed radiotracer kinetics in the left lower extremity. Due to no clinical sings of edema on the left leg, this scintigraphic findings were important for visualization of dysfunction. The sensitivity of qualitative lymphoscintigraphy in the diagnosis of lymphedema is 70% [16]. Some studies claim that the sensitivity of qualitative lymphoscintigraphy in the diagnosis of lymphedema is 96% and the specificity 100% in centers that have years of experience in the diagnosis of lymphedema; however, lower sensitivity has been previously reported due to lack of knowlede about the diseases that lead to limb swelling and some studies included patients who would not be considered to have lymphedema according to today's clinical criteria It is also extremely important to note that the clinical stage of lymphedema does not correlate with lymphoscintigraphic findings, which means that patients with severe lymphedema may have delayed transit of radiotracer or that patients with clinically mild lymphedema may have markedly delayed transit in the region of lymph nodes [23]. There are also papers suggesting that quantitative data obtained by measuring radioactive decay are often inconsistent or that it is often not possible to adequately perform such measurements [24]. We have already discussed the pathological findings of qualitative lymphoscintigraphy, however some authors believe that the lym-

phoscintigraphic findings are different in primary and secondary lymphedemas [14], but most studies claim that these two entities cannot be distinguished by lymphoscintigraphy [18]. Some studies report that primary lymphedema is lymphoscintigraphically characterized by delayed or absent transport of radiotracer or absence of lymphatic pathways followed by poor visualization or lack of regional lymph nodes and occasional dermal backflow on early imaging, but this finding may correspond to primary lymphedema when there is no clinical data suggesting lymphedema of secondary cause. The lymphoscintigram in patients with secondary lymphedema shows dilated lymphatic pathways, collateral lymphatic pathways, lymphatic pathway disruption, delayed transport of radiotracer, and dermal backflow on delayed imaging [25].

Lymphoscintigraphy may be useful preoperatively; Vaqueiro et al. point to the benefit of lymphoscintigraphy in the selection of patients for microvascular procedures, lymphatic-venous anastomosis, by showing the passabel lymphatic pathways that are suitable for making an anastomosis [26].

These types of surgeries have recently gained more popularity and are most effective in the early stages of the disease and in patients with secondary lymphedema compared to patients with primary lymphedema. This is because in patients with primary lymphedema the lymph vessels are structurally damaged and cannot be used as good permeable grafts. These surgeries have so far shown better results on the upper extremities compared to the lower extremities [27].

Lee and Bergan emphasize the use of lymphoscintigraphy in predicting the outcome of lymphedema treatment. They devised a lymphedema grading system based on lymphoscintigraphic findings and used it with the clinical lymphedema grading system to predict the outcome of treatment as well as to determine if additional drug or surgical treatment of lymphedema is needed [28].

Although newer imaging methods may provide additional information on extremity lymphedema, which may be useful in planning surgery [29], they are not as accurate in the diagnosis of lymphedema. Magnetic resonance lymphangiography outlines the lymphatic vessels of the extremities but its sensitivity is 68% in the diagnosis of lymphedema [30]. Lymphangiography with indocyanine highlights subdermal lymphatic pathways, but its specificity in the diagnosis of lymphedema is 55% [31].

Conclusion

Lymphoscintigraphy is reliable in the diagnosis of lymphedema with a sensitivity of up to 96% and a specificity of 100%. The procedure itself is practically painless and requires no special preparation of patients. Lymphoscintigraphy has proven to be a superior method in the diagnosis of lymphedema compared to other diagnostic methods. It is of great importance that this procedure can be repeated several times in one patient in order to monitor the condition after the applied therapeutic modalities, all without fear of additional damage to the lymphatic system. This method can be used preoperatively, in the selection of patients for formation of lymphatic venous anastomosis, as well as a tool for predicting the treatment outcome of patients with lymphedema.

References

1. Creager MA, Loscalzo J. Chronic venous disease and lymphedema. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, editors. Harrison's principles of internal medicine. 20th ed. New York: McGraw-Hill Education; 2018. p. 1930-5.

2. Ahmed RL, Prizment A, Lazovich D, Schmitz KH, Folsom AR. Lymphedema and quality of life in breast cancer survivors: the Iowa Women's Health Study. J Clin Oncol. 2008;26(35):5689-96.

3. Rockson SG, Rivera KK. Estimating the population burden of lymphedema. Ann N Y Acad Sci. 2008;1131:147-54.

4. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. Lancet Oncol. 2013;14(6):500-15.

5. Brouillard P, Boon L, Vikkula M. Genetics of lymphatic anomalies. J Clin Invest. 2014;124(3):898-904.

6. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology. Lymphology. 2013;46(1):1-11.

7. Cambria RA, Gloviczki P, Naessens JM, Wahner HW. Noninvasive evaluation of the lymphatic system with lymphoscintigraphy: a prospective, semiquantitative analysis in 386 extremities. J Vasc Surg. 1993;18(5):773-82.

8. Partsch H. Assessment of abnormal lymph drainage for the diagnosis of lymphedema by isotopic lymphangiography and by indirect lymphography. Clin Dermatol. 1995;3(5):445-50.

9. Ter SE, Alavi A, Kim CK, Merli G. Lymphoscintigraphy. A reliable test for the diagnosis of lymphedema. Clin Nucl Med. 1993;18(8):646-54.

10. Szuba A, Shin WS, Strauss HW, Rockson S. The third circulation: radionuclide lymphoscintigraphy in the evaluation of lymphedema. J Nucl Med. 2003;44(1):43-57.

11. Strand SE, Bergqvist L. Radiolabeled colloids and macromolecules in the lymphatic system. Crit Rev Drug Carrier Syst. 1989;6(3):211-38.

12. Hung JC, Wiseman GA, Wahner HW, Mullan BP, Taggart T, Dunn WL. Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy. J Nucl Med. 1995;36(10):1895-901.

13. Williams WH, Witte CL, Witte MH, McNeill GC. Radionuclide lymphangioscintigraphy in the evaluation of peripheral lymphedema. Clin Nucl Med. 2000;25(6):451-64.

14. Campisi CC, Ryan M, Villa G, Di Summa P, Cherubino M, Boccardo F, et al. Rationale for study of the deep subfascial lymphatic vessels during lymphoscintigraphy for the diagnosis of peripheral lymphedema. Clin Nucl Med. 2019;44(2):91-8.

15. Szuba A, Rockson SG. Lymphedema: classification, diagnosis and therapy. Vasc Med. 1998;3(2):145-56.

16. Weissleder H, Weissleder R. Lymphedema: evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. Radiology. 1988;167(3):729-35.

17. Sadeghi R, Kazemzadeh G, Keshtgar M. Diagnostic application of lymphoscintigraphy in the management of lymphoedema. Hell J Nucl Med. 2010;13(1):6-10.

18. Tomczak H, Nyka W, Lass P. Lymphoedema: lymphoscintigraphy versus other diagnostic techniques-a clinician's point of view. Nucl Med Rev Cent East Eur. 2005;8(1):37-43.

19. Pecking AP. Possibilities and restriction of isotopic lymphography for the assessment of therapeutic effects in lymphedema. Wien Med Wochenschr. 1999;149(2-4):105-6.

20. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema. Consensus document of the International Society of Lymphology. Lymphology. 2003;36(2):84-91.

21. EBM guidelines on the diagnosis and treatment of lymphedema. European Journal of Lymphology and Related Problems. 2006;16(46):11-21.

22. Hassanein AH, Maclellan RA, Grant FD, Greene AK. Diagnostic accuracy of lymphoscintigraphy for lymphedema and analysis of false-negative tests. Plast Reconstr Surg Glob Open. 2017;5(7):e1396.

23. Maclellan RA, Zurakowski D, Voss S, Greene AK. Correlation between lymphedema disease severity and lymphoscintigraphic findings: a clinical-radiologic study. J Am Coll Surg. 2017;225(3):366-70.

24. Jensen MR, Simonsen L, Karlsmark T, Bülow J. The washout rate of a subcutaneous 99mTc-HSA depot in lower extremity lymphoedema. Clin Physiol Funct Imaging. 2012;32(2):126-32.

25. Scarsbrook AF, Ganeshan A, Bradley KM. Pearls and pitfalls of radionuclide imaging of the lymphatic system. Part 2: evaluation of extremity lymphoedema. Br J Radiol. 2007;80 (951):219-26.

26. Vaqueiro M, Gloviczki P, Fisher J, Hollier LH, Schirger A, Wahner HW. Lymphoscintigraphy in lymphedema: an aid to microsurgery. J Nucl Med. 1986;27(7):1125-30.

27. Garza RM, Chang DW. Lymphovenous bypass for the treatment of lymphedema. J Surg Oncol. 2018;118(5):743-9.

28. Lee BB, Bergan JJ. New clinical and laboratory staging systems to improve management of chronic lymphedema. Lymphology. 2005;38(3):122-9.

29. Chang DW, Masia J, Garza R, 3rd, Skoracki R, Neligan PC. Lymphedema: surgical and medical therapy. Plast Reconstr Surg. 2016;138(3 Suppl):209S-18.

30. Weiss M, Burgard C, Baumeister R, Strobl F, Rominger A, Bartenstein P, et al. Magnetic resonance imaging versus lymphoscintigraphy for the assessment of focal lymphatic transport disorders of the lower limb: first experiences. Nuklearmedizin. 2014;53(5):190-6.

31. Akita S, Mitsukawa N, Kazama T, Kuriyama M, Kubota Y, Omori N, et al. Comparison of lymphoscintigraphy and indocyanine green lymphography for the diagnosis of extremity lymphoedema. J Plast Reconstr Aesthet Surg. 2013;66(6):792-8.

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MENTAL HEALTH OF PATIENTS WITH CHRONIC DISEASES DURING THE CORONAVIRUS DISEASE 2019 PANDEMIC IN SERBIA − A CROSS-SECTIONAL STUDY

MENTALNO ZDRAVLJE LJUDI SA HRONIČNIM BOLESTIMA TOKOM COVID-19 PANDEMIJE U SRBIJI − STUDIJA PRESEKA

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Summary

Introduction. Since the coronavirus disease 2019 outbreak was declared a pandemic by the World Health Organization on March 11, 2020, it has become the greatest public health threat worldwide. People with chronic diseases were identified as the group at risk for fatal outcome. The purpose of this research is to estimate the mental health of patients with chronic diseases during the coronavirus disease 2019 pandemic. **Material and Methods.** A total of 463 individuals (50.8% female), with the average age of 46.63 years (SD = 14.29, ranging from 20 to 75 years of age) participated in the research. The subjects were divided into two groups, based on the existence of at least one chronic disease. The Depression, Anxiety and Stress Scale-21 was used to assess the mental health of the participants. The research was conducted in August 2020. The impact of chronic illness on depression, anxiety, and stress levels was assessed using linear regression models. **Results.** The prevalence of chronic diseases among the participants was 44.3%. The participants with chronic diseases presented with higher levels of depression ($p < 0.05$), anxiety ($p < 0.001$), and stress ($p < 0.001$) compared to healthy participants. The presence of chronic illness remains a significant predictor of all the dependent variables, even after the inclusion of multiple variables in the final regression model: depression (Beta [β] 0.37; 95% confidence interval: 2.67 – 4.42; $p < 0.01$), anxiety (Beta [β] 0.19; 95% confidence interval: 0.80 – 2.55; $p < 0.01$), and stress (Beta [β] 0.09; 95% confidence interval: 0.01 – 2.13; $p < 0.05$). **Conclusion.** About five months after the coronavirus disease 2019 pandemic was declared, the investigation of mental health of chronically ill adults in Serbia shows an increased amount of stress, anxiety, and depression in this subpopulation.

Key words: Mental Health; Chronic Disease; Pandemics; Coronavirus Infections; Depression; Anxiety; Stress, Psychological; Surveys and Questionnaires

Sažetak

Uvod. Od kada je 11. marta 2020. godine Svetska zdravstvena organizacija proglasila COVID-19 pandemiju, ona je postala najveća pretnja globalnom javnom zdravlju. Kao rizična grupa za nastanak smrtnog ishoda identifikovane su osobe sa hroničnim bolestima. Cilj ovog istraživanja bio je procena mentalnog zdravlja ljudi sa hroničnim bolestima tokom pandemije COVID-19. **Materijali i metode.** U istraživanju je učestvovalo 463 ispitanika (50,8% ženskog pola), prosečne starosti 46,63 godina (SD = 14,29, raspon od 20 do 75 godina). Ispitanici su podeljeni u dve grupe na osnovu postojanja makar jedne hronične bolesti. Skala 21 depresivnosti, anksioznosti i stresa korišćena je za procenu mentalnog zdravlja odraslog stanovništva u Srbiji. Istraživanje je sprovedeno tokom avgusta 2020. Kroz niz linearnih regresionih modela procenjen je uticaj hroničnih bolesti na nivo depresivnosti, anksioznosti i stresa. **Rezultati.** Učestalost hroničnih bolesti u uzorku iznosi 44,3%. Kod ispitanika sa hroničnim bolestima beleže se viši nivoi depresije ($p < 0,05$), anksioznosti ($p < 0,001$) i stresa ($p < 0,001$) u odnosu na zdrave ispitanike. Prisustvo hroničnih bolesti ostalo je značajan prediktor svih zavisnih varijabli i nakon uključivanja više varijabli u konačan regresioni model: depresivnost (beta [β] 0,37; 95% interval pouzdanosti: 2,67 – 4,42; $p < 0,01$), anksioznost (beta [β] 0,19; 95% interval pouzdanosti: 0,80–2,55; $p < 0,01$) i stres (beta [β] 0,09; 95% interval pouzdanosti: 0,01–2,13; $p < 0,05$). **Zaključak.** Približno pet meseci od proglašenja COVID-19 pandemije, istraživanje mentalnog zdravlja u Srbiji na odraslim osobama sa hroničnim bolestima pokazuje povećanu količinu stresa, anksioznosti i depresije kod ove subpopulacije.

Ključne reči: mentalno zdravlje; hronično oboljenje; pandemija; koronavirus infekcija; depresija; anksioznost; stres; istraživanja i upitnici

Introduction

The psychological reality of physical illness is the patient's subjective construction. For any lifelong illness, the question of its impact on the person's mental health inevitably gets raised. Can the disabled, or those who live with a death threat every day, be expected to respond to the stress of a pandemic unfolding around them with the same resilience as healthy persons?

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Abbreviations

COVID-19 – coronavirus disease 2019
DASS-21 – Depression, Anxiety and Stress Scale-21
CVD – cardiovascular disease
DM – diabetes mellitus
CI – confidence interval

Chronic noncommunicable diseases are globally the leading cause of death with 41 million deaths per year [1]. Over 70% of all premature deaths worldwide are caused by cardiovascular disease (CVD) (17.9 million deaths per year), malignant diseases (9 million deaths per year), respiratory diseases (3.9 million deaths per year), and diabetes mellitus (DM) (1.6 million deaths per year) [1].

In December 2019, an outbreak of the new coronavirus pneumonia appeared in Wuhan, Hubei Province, China [2] and it quickly spread becoming a global menace. The coronavirus disease 2019 (COVID-19) outbreak was declared a pandemic by the World Health Organization on March 11, 2020. Approximately 25,602,665 confirmed cases of the COVID-19 have been reported worldwide, including 852,758 deaths [3]. Studies have been conducted to examine the impact of COVID-19 on chronically ill persons. Analysis of 72,314 cases in China established the case fatality ratio (CFR) to be 10.5% for CVB, 7.3% for DM, 6.3% for chronic respiratory syndrome, and 6% for hypertension, while the CFR for the general population was 2.3% [4]. A study conducted in Italy also shows that the presence of comorbidities may raise the risk of death in COVID-19 cases [5]. The elderly suffering from chronic diseases such as DM, CVD, hypertension, asthma, or stroke were found to be at highest risk [5].

Numerous studies have shown that living with a chronic disease affects the person's mental health. For example, in cerebrovascular diseases, there is permanent damage to the brain tissue caused by a somatic disorder; coronary heart disease affects the severity of depressive symptoms in surviving apoplexy [6], and the removal of the vascular pathology substrate often leads to improved cognitive function and reduced depression [7]; depression often develops as part of type-2 DM and has been identified as one of the factors that accelerates the onset of advanced degenerative changes to small blood vessels. Patients on hemodialysis face a number of difficulties brought on by the nature of the disease. This contributes significantly to the onset of depression which is the most common psychological disorder afflicting these individuals [8]. Depression and depressive disorders are often associated with numerous sociodemographic factors. A study conducted by Vučurević et al. found an association between depression and marital status, employment, previous contact with mental health services, but above all with chronic illness [9]. Anxiety is experienced by all dialysis patients examined in a study conducted by Novaković et al. in 2007 [10].

Generally speaking, psychological reactions to a somatic disease can be manifested through the following [11]:

Anxiety (concern over disease prognosis - uncertainty can be harder to cope than the worst prognosis);

Regression (dependence on others - at the level of a child, with attachment to the mother; passivity - receptive personality structure - the position of a child);

Depression (people in whom the super-ego is rigid and who function on the principle of perfection);

Aggression (anger at and rage against the environment - projection of feelings of dissatisfaction onto others)

Retroflexion - self-aggression, increased risk of suicide.

The advent of the COVID-19 has dramatically changed the lives of people worldwide. Measures such as quarantine, self-isolation, ban on movement, traffic suspension, and working from home, have become our new daily routine. Disasters, such as a pandemic, can and do undermine people's feelings of security, reminding them of their own and the mortality of their loved ones, adversely affecting their mental health. No answers are in sight to questions raised as humanity faces the ongoing COVID-19 pandemic, such as when it will end, or what the consequences for society will be; effective treatment methods remain lacking; people are constantly exposed to controversial, even contradictory information; social relations are impaired, and drastic measures, such as quarantine, introduced - all this can potentially be detrimental to the mental health of individuals. Symptoms such as anxiety, depression, fear, stress, and sleep problems tend to become more pronounced during pandemics, but the effects of this particular pandemic on the mental health of the chronically ill are in need of further research.

The purpose of this study is to evaluate the levels of depression, anxiety and stress in the chronically ill in Serbia at the time of the pandemic wave, in the summer of 2020. We also wish to determine the state of the mental health of certain socioeconomic groups during the COVID-19 pandemic.

The main objective of this paper is to investigate distress, anxiety, and depression levels of adults with chronic diseases at the time of the COVID-19 pandemic in Serbia, and the differences in these parameters between healthy individuals and those chronically ill.

It is important to investigate the pandemic's psychological impact on particular groups in order to develop strategies toward mitigating both mental and physical ailments of individuals during this time. We expect that the chronically ill individuals will experience higher levels of stress, anxiety, and depression. We also expect that vulnerable groups, such as women, seniors, single parents, and persons of low income, will all have higher levels of the above-mentioned symptoms.

Several studies have investigated the impact of COVID-19 on the mental health of the chronically ill, such as Özdin et al. [12], Louvardi et al. [13], and Ozamiz-Etxebarria et al. [14], and they all came to similar conclusions – persons suffering from chronic diseases have more pronounced mental health issues during the pandemic.

Table 1. Sample characteristics in regard to health status
Tabela 1. *Karakteristike uzorka s obzirom na zdravstveni status ispitanika*

	Chronically ill respondents *Ispitanici sa hroničnim bolestima* (n=205)	Healthy respondents *Zdravi ispitanici* (n=258)	p *p*	All respondents *Svi ispitanici* (n=463)
Gender/*Pol*, *n (%)*				
Male/*Muški*	87 (42.4%)	141 (54.7%)	0.009 [a]	228 (49.2%)
Female/*Ženski*	118 (57.6%)	117 (45.3%)		235 (50.8%)
Age, M ± SD (Min – Max) *Starost, prosek ± SD (Min. – Maks.)*	54.32±12,36 (20-75)	40.53 ±12,71 (20-74)	0.000 [b]	46.63± 14,29 (20-75)
Age category (years)/*Starosne kategorije (godine), n (%)*				
20 – 44	45 (22.0%)	167 (64.7%)		212 (45.8%)
45 – 64	119 (58.0%)	79 (30.6%)	0.000 [a]	198 (42.8%)
≥ 65	41 (20.0%)	12 (4.7%)		53 (11.4%)
Education (years)/*Obrazovanje (godine), n (%)*				
≤ 8	31 (16.0%)	13 (5.2%)		44 (9.9%)
8 – 12	108 (55.7%)	146 (58.6%)	0.001 [a]	254 (57.4%)
≥ 12	55 (28.3%)	90 (36.2%)		145 (32.7%)
Employment status/*Radni status, n (%)*				
Student/*Student*	5 (2.5%)	18 (7.0%)		23 (5.0%)
Unemployed/*Nezaposlen/a*	14 (6.9%)	49 (19.1%)		63 (13.7%)
Employed in private sector *Zaposlen/a u privatnom sektoru*	60 (29.7%)	88 (34.2%)	0.000 [a]	148 (32.2%)
Employed in public sector *Zaposlen/a u državnom sektoru*	21 (10.4%)	62 (24.1%)		83 (18.1%)
Farmer/homemaker/*Poljoprivrednik/domaćica*	17 (8.4%)	19 (7.4%)		36 (7.8%)
Retired/*Penzioner/ka*	85 (42.1%)	21 (8.2%)		106 (23.2%)
Family material status/*Imovinsko stanje porodice, n (%)*				
Below average/*Ispod proseka*	20 (14.1%)	7 (4.5%)		27 (9.1%)
Average/*U proseku*	107 (75.4%)	117 (76.0%)	0.004 [a]	224 (75.7%)
Above average/*Iznad proseka*	15 (10.5%)	30 (19.5%)		45 (15.2%)
Marital status/*Bračni status, n (%)*				
Married/*Oženjen/udata*	136 (68.3%)	163 (64.9%)		299 (66.4%)
Single/*Neoženjen/neudata*	24 (12.1%)	64 (25.5%)	0.000 [a]	88 (19.6%)
Divorced/*Razveden/a*	13 (6.5%)	15 (6.0%)		28 (6.2%)
Widowed/*Udovac/udovica*	26 (13.1%)	9 (3.6%)		35 (7.8%)
Type of current residence/*Mesto stanovanja, n (%)*				
Urban/*Grad*	104 (50.7%)	141 (54.7%)	0.401 [a]	245 (52.9%)
Rural/*Manje mesto/selo*	101 (49.3%)	117 (45.3%)		218 (47.1%)
Types of diseases/*Vrsta hronične bolesti, n (%)*				
CVD/*Kardiovaskularne bolesti*	151 (73.7%)	/		151 (32.6%)
Musculoskeletal disorders/*Muskuloskeletne bolesti*	49 (23.9%)	/		49 (10.6%)
Respiratory disorders/*Respiratorne bolesti*	38 (18.5%)	/	/	38 (8.2%)
DM/*Dijabetes melitus*	24 (11.7%)	/		24 (5.2%)
Others/*Druge hronične nezarazne bolesti*	27 (13.2%)	/		27 (5.8%)
Number of chronic diseases/*Broj hroničnih bolesti, n (%)*				
0	0 (0.0%)	258 (100%)		258 (55.7%)
1	133 (64.8%)	0 (0.0%)	/	133 (28.7%)
2	61 (29.8%)	0 (0.0%)		61 (13.2%)
≥ 3	11 (5.4%)	0 (0.0%)		11 (2.4%)

Legend**:** [a] Chi-square test (X^2); [b] Independent Samples T-Test; M±SD (Min – Max) - Mean±Std. Deviation (Minimum – Maximum); p - Statistical significance; n - Number of respondents;
Legenda: *[a] Hi kvadrat test (X^2); [b] T-test za velike nezavisne uzorke; M ± SD (Min. – Maks.) – aritmetička sredina ± standardna devijacija (minimum – maksimum); p – statistička značajnost; n – broj ispitanika*

Material and Methods

This cross-sectional study aimed to assess the mental health of the general adult population in Serbia at the time of the COVID-19 epidemic and also to examine whether people with chronic diseases are in a disadvantaged position. The study was conducted via an online panel, where the participants anonymously filled out a questionnaire over a period of five days (August 10 – 15, 2020). The participants were asked to fill out a questionnaire, the beginning of which disclosed the title and purpose of the research, as well as the time necessary to complete it. Filling out the questionnaire took about ten minutes. A total of 463 individuals participated in the study, with a confidence level of 95% and confidence interval (CI) of 4, the calculated sample size being 435 persons. The calculations were done using the G*Power software. The participants were all twenty years of age or older (exclusion criteria: < 20 years of age).

The online questionnaire covered several areas: (a) general data, (b) chronic disease data, and (c) Depression, Anxiety and Stress Scale-21 (DASS-21).

The general data referred to socio-demographic variables: gender (male/female), age (open question), education level (≤ 8 years/8 – 12 years/≥ 12 years), employment status (student/unemployed/employed in the private sector/employed in the public sector/farmer/homemaker/retired), family material status (below average/average/above average), marital status (married/single/divorced/widowed), and type of current residence (urban/rural).

The chronic illness data were given by the participants themselves. The question was: "In the last 12 months, did you have one of the following diseases or conditions?" A list of chronic diseases was provided for the participants to select from, in case they suffered from any of the listed. Data collection on the prevalence of chronic illness was conducted in accordance with the European Health Interview Survey recommendations [15]. The diseases were subsequently separated into five categories: CVD, respiratory disorders, musculoskeletal disorders, DM, and others. The respondents were also subsequently divided into two groups - chronically ill individuals (those with at least one chronic disease) and healthy individuals (those without chronic diseases).

The DASS-21 was used to estimate the mental condition of the adult population of Serbia at the time of the COVID-19 pandemic. It consists of three self-report scales that measure depression, anxiety, and stress. All subscales are rated on a four-point Likert scale ranging from 0 (never) to 3 (almost always). The DASS-21 is translated and adapted for the Serbian population [16]. In this study, the reliability of the depression, anxiety, and stress subscales was 0.84, 0.79, and 0.82, respectively. It has been used in other studies in the COVID-19 pandemic period, showing good reliability [17, 18].

Descriptive analysis was performed on the socio-demographic characteristics, health status, and DASS-21 subscales. The obtained results were presented using frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. We assessed the difference using the Independent samples t-test. Analysis of variance (ANOVA) was used for comparison of more than two independent samples. The chi-square test was used to assess the differences between categorical variables. To examine the impact of chronic illness on the DASS-21 subscale, we performed a series of linear regression models based on potential confounding effects of the other observed variables. The DASS-21 subscales, depression, anxiety, and stress, were used as dependent variables. Three regression models were used for all three dependent variables. The first model, Chronic Illness Model, is a univariate linear regression model. In the Basic Model, age and gender were added as covariates. In the Full Model, we included level of education, employment status, family material status, marital status, and type of current residence as covariates. The effect estimates were presented as Beta coefficients, with a corresponding 95% CI. The probability level of $p \leq 0.05$ was considered statistically significant. Statistical analysis was carried out using the Statistical package for the social sciences (SPSS) for Windows, ver. 24.0 (IBM Corp., Armonk, NY, USA).

Results

Table 1 shows the socio-demographic characteristics of the two subsamples. As per their own testimony, 205 (44.3%) adult respondents over the age of 20 had at least one chronic illness. Cardiovascular diseases were the most common, followed by musculoskeletal diseases, respiratory diseases, and DM, at 32.6%, 10.6%, 8.2%, and 5.2%, respectively. Of the total number of respondents with chronic illness, 64.9% reported having one chronic illness, 29.8% reported having two, while three or more chronic diseases were reported by 5.4% of the respondents.

The average values of the three DASS-21 subscales of the questionnaire for both the chronically ill and healthy respondents are shown in **Graph 1**. Depression is more pronounced in chronically ill respondents than in the healthy (7.03 [SD = 5.15] *vs.* 3.29 [SD = 3.51]; $p < 0.05$). Anxiety was also higher in the chronically ill (4.81 [SD = 4.75] *vs.* 3.30 [SD = 3.78]; $p < 0.001$), as was stress (7.29 [SD = 5.39] *vs.* 6.03 [SD = 5.33]; $p < 0.001$).

The average values of depression, anxiety, and stress in the socio-demographic groups of respondents are also presented **(Table 2)**. Higher subscale values of depression were reported by women, elderly respondents, those with elementary education, low income respondents, and widowers. Depression was also more pronounced in those with all types of chronic diseases. Higher anxiety was reported by women, the unemployed, and those with CVD and musculoskeletal diseases. During the COVID-19 pandemic, higher levels of stress were experienced by women, the unemployed, married, as well as by those with respiratory and musculoskeletal diseases.

Table 2. Mean scores on the DASS-21 scales among the socio-demographic groups
Tabela 2. *Prosečne vrednosti na Skali 21 depresivnosti, anksioznosti i stresa kod ispitanika sa različitim socio-demografskim karakteristikama*

	Depression *Depresija*	p *p*	Anxiety *Anksioznost*	p *p*	Stress *Stres*	p *p*
Gender/*Pol*, *n (%)*						
Male/*Muški*	3.73±3.99	0.000 [a]	3.08±3.76	0.000 [a]	4.61±5	0.000 [a]
Female/*Ženski*	6.13±5.03		4.84±4.62		8.51±5.06	
Age category (years)/*Starosne kategorije (godine), n (%)*						
20 – 44	4.17±4.47		3.88±4.2		6.19±5.24	
45 – 64	5.41±4.52	0.002 [b]	3.85±4.25	0.336 [b]	6.96±5.53	0.341 [b]
≥ 65	6.34±5.66		4.79±4.87		6.79±5.42	
Education (years)/*Obrazovanje (godine)*, *n (%)*						
≤ 8	7.23±6.63		4.86±4.93		6.89±5.62	
8 – 12	4.9±4.7	0.002 [b]	3.87±4.16	0.341 [b]	6.77±5.32	0.433 [b]
≥ 12	4.37±3.97		3.84±4.4		6.08±5.43	
Employment status/*Radni status, n (%)*						
Student/*Student*	2±2.89		2.65±2.71		4.57±4.44	
Unemployed/*Nezaposlen/a*	6.25±5.86		6.29±4.58		8.76±5.41	
Employed in private sector/*Zaposlen/a u privatnom sektoru*	4.37±3.85	0.000 [b]	3.05±3.83	0.000 [b]	5.91±5.32	0.002 [b]
Employed in public sector/*Zaposlen/a u državnom sektoru*	4.11±4.28		3.14±3.65		6±5.31	
Farmer/homemaker/*Poljoprivrednik/domaćica*	4.31±3.5		3.83±3.75		5.86±5.02	
Retired/*Penzioner/ka*	6.42±5.28		4.84±4.94		7.28±5.45	
Family material status/*Imovinsko stanje porodice, n (%)*						
Below average/*Ispod proseka*	9.7±6.98		5.11±4.73		7.7±6.7	
Average/*U proseku*	4.74±4.27	0.000 [b]	3.67±4.4	0.062 [b]	6.58±5.6	0.064 [b]
Above average/*Iznad proseka*	3.62±3.9		2.64±3.36		4.78±4.45	
Marital status/*Bračni status, n (%)*						
Married/*Oženjen/udata*	4.9±4.45		4±4.31		7.09±5.43	
Single/*Neoženjen/neudata*	3.76±4.26	0.000 [b]	3.39±3.91	0.248 [b]	4.98±5.05	0.015 [b]
Divorced/*Razveden/a*	4.79±4.26		3.96±4.57		6.68±5.5	
Widowed/*Udovac/udovica*	8.17±6.49		5.11±4.74		6.54±5.5	
Type of current residence/*Mesto stanovanja, n (%)*						
Urban/*Grad*	4.77±4.67	0.380 [a]	3.96±4.24	0.929 [a]	6.83±5.33	0.312 [a]
Rural/*Manje mesto/selo*	5.15±4.73		3.99±4.39		6.32±5.45	
CVD/*Kardiovaskularne bolesti*						
No/*Ne*	3.96±4.11	0.000 [a]	3.62±3.97	0.010 [a]	6.40±5.38	0.279 [a]
Yes/*Da*	7.00±5.17		4.70±4.85		6.98±5.42	
DM/*Dijabetes*						
No/*Ne*	4.83±4.63	0.000 [a]	3.94±4.28	0.446 [a]	6.60±5.39	0.841 [a]
Yes/*Da*	7.17±5.41		4.63±4.81		6.38±5.44	
Respiratory disorders/*Respiratorne bolesti*						
No/*Ne*	4.75±4.61	0.000 [a]	3.96±4.27	0.842 [a]	6.40±5.33	0.012 [a]
Yes/*Da*	7.18±5.11		4.11±4.72		8.68±5.67	
Musculoskeletal disorders/*Muskuloskeletne bolesti*						
No/*Ne*	4.68±4.63	0.000 [a]	3.76±4.13	0.002 [a]	6.39±5.35	0.020[a]
Yes/*Da*	7.20±4.71		5.80±5.26		8.29±5.45	
Others/*Druge hronične nezarazne bolesti*						
No/*Ne*	4.81±4.65	0.000 [a]	3.90±4.28	0.170[a]	6.60±5.48	0.857[a]
Yes/*Da*	7.22±4.95		5.07±4.67		6.41±3.61	
Number of chronic illnesses/*Broj hroničnih bolesti, n (%)*						
1	6.89±5.32		4.82±4.69		7.22±5.43	
2	7.38±4.88	0.821[b]	4.82±4.57	0.998[b]	7.51±5.51	0.915[b]
≥ 3	6.82±4.96		4.73±6.75		6.91±4.68	

Legend**:** [a]Independent Samples T-Test; [b]One-way analysis of variance (ANOVA); p - Statistical significance; n - Number of respondents; Note: Mean ± Std. Deviations are shown in the table
Legenda: [a] T-test za velike nezavisne uzorke; [b]Jednofaktorska analiza varijanse (ANOVA); p – statistička značajnost; n – broj ispitanika; Napomena: u Tabeli je prikazana aritmetička sredina ± standardna devijacija

Table 3 shows the linear regression models with the DASS-21 subscale of depression as the dependent variable. In all models, the presence of chronic illness was significantly associated with worsened depression. In the univariate linear regression model, chronic illness was significantly associated with worsened depression (Beta [β] 0.39; 95% CI: 2.94 – 4.52; $p < 0.01$). After adjustment for gender and age in the Basic Model, the presence of chronic illness remained associated with worsened depression (Beta [β] 0.36; 95% CI: 2.55 – 4.33; $p < 0.01$). Adjusting for multiple confounding factors in the Full Model, chronic illness was still found to be associated with worsened depression (Beta [β] 0.37; 95% CI: 2.67 – 4.42; $p < 0.01$).

Similar results were obtained using the regression models when anxiety was the dependent variable. When all the variables were included as confounding factors in the Full Model, chronic illness remained a statistically significant predictor of anxiety (Beta [β] 0.19; 95% CI: 0.80 – 2.55; $p < 0.01$) **(Table 4)**.

The presence of chronic illness was also found to be significantly associated with worsened stress levels in all regression models. After adjusting for all variables in the Full Model, chronic illness was still found to be significantly related to higher levels of stress (Beta [β] 0.09; 95% CI: 0.01 – 2.13; $p < 0.05$) **(Table 5)**.

Discussion

The prevalence of chronic disease in the adult population of Serbia in our sample was 44.3%. Among the chronically ill participants, CVD were most common, followed by musculoskeletal diseases, respiratory diseases, DM, and others: 73.7%, 23.9%, 18.5%, 11.7%, 13.2%, respectively. Many respondents (35.2%) reported more than one illness. As per their own testimony, more than half (53.5%) of the people over the age of 15 living in Serbia in 2003 had at least one chronic illness [19].

The average values of depression, anxiety, and stress were all higher to a statistically significant extent in the group of chronically ill participants (7.03 [SD = 5.15], 4.81 [SD=4.75] and 7.29 [SD = 5.39], respectively) than in the healthy group (3.29 [SD = 3.51], 3.30 [SD = 3.78] and 6.03 [SD = 5.33], respectively). In a study includig 1,374 students in Serbia prior to the COVID-19 pandemic, the average level of depression was 3.14 (SD = 3.90), anxiety 3.37 (SD = 3.82), and stress 6.70 (SD = 11.01) [16]. Mental health is thus worse in the group of chronically ill participants. In all of the regression models, chronic illness proved a statistically significant predictor of a higher level of depression, anxiety, and

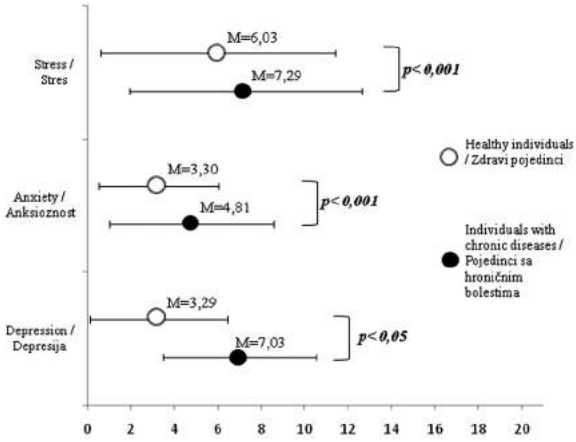
Table 3. Linear regression models of the association between health status and depression
Tabela 3. *Linearni regresioni modeli povezanosti zdravstvenog statusa ispitanika i depresije*

	Chronic illnesses model *Model sa hroničnim bolestima*	Basic model *Osnovni model*	Full model *Kompletan model*
Presence of chronic diseases (ref.: no diseases)/*Prisustvo hroničnih bolesti (ref.: bez prisustva bolesti)*			
Yes/*Da*	0.39 (2.94 - 4.52)**	0.36 (2.55 - 4.33)**	0.37 (2.67 - 4.42)**
Gender (ref.: males)/*Pol (ref.: muški pol)*			
Females/*Žene*		0.21 (1.20 - 2.75)**	0.20 (1.13 - 2.66)**
Age/*Starost*		0.01 (-0.02 - 0.03)	0.02 (-0.03 - 0.05)
Education (ref.: ≥12 years)/*Obrazovanje (ref.: ≥12 godina)*			
≤ 8			0.06 (-0.39 - 2.57)
8 – 12			0.01 (-0.70 - 0.97)
Employment status (ref.: student)/*Radni status (ref.: student)*			
Unemployed/*Nezaposlen/a*			0.26 (1.61 - 5.53)**
Employed in private sector *Zaposlen/a u privatnom sektoru*			0.12 (-0.78 - 3.22)
Employed in public sector *Zaposlen/a u državnom sektoru*			0.12 (-0.57 - 3.60)
Farmer/homemaker/*Poljoprivrednik/domaćica*			0.02 (-1.83 - 2.72)
Retired/*Penzioner/ka*			0.03 (-2.00 - 2.86)
Family material status (ref.: above average)/*Imovinsko stanje porodice (ref.: iznad proseka)*			
Below average/*Ispod proseka*			0.16 (1.46 - 5.04)**
Average/*Prosečno*			0.01 (-0.80 - 1.02)
Marital status (ref.: widowed)/*Bračni status (ref.: udovac/udovica)*			
Married/*Oženjen/udata*			-0.12 (-2.53 - 0.11)
Single/*Neoženjen/neudata*			-0.12 (-3.24 - 0.17)
Divorced/*Razveden/a*			-0.03 (-2.63 - 1.32)
Type of current residence (ref.: rural)/*Mesto stanovanja (ref.: manje mesto/selo)*			
Urban/*Grad*			-0.01 (-0.93 - 0.59)

Note: Values represent Beta coefficients with corresponding 95% confidence intervals; *$p < 0.05$; **$p < 0.01$
*Napomena: Vrednosti predstavljaju beta koeficijente sa 95% intervalom poverenja; *$p < 0.05$; **$p < 0.01$*

stress. A study conducted in Greece from March to May 2020, including 943 healthy persons and 163 chronically ill persons, shows that the chronically ill had significantly higher levels of distress (p = 0.001), but there was no significant difference in anxiety (p = 0.098) or depression (p = 0.052) [13]. A more recent Chinese study shows that the chronically ill have higher levels of stress [18].

Higher levels of depression were reported by respondents with CVD, DM, respiratory diseases, musculoskeletal diseases, and other chronic diseases compared to health respondents. Compared to healthy participants, higher anxiety was reported by those with cardiovascular and musculoskeletal diseases, while respondents with respiratory and musculoskeletal diseases reported more pronounced levels of stress. The estimate of stress prevalence among diabetics was 49.2%, 1.47 times higher than in the healthy controls, according to a study conducted in Bangladesh during the COVID-19 pandemic [20]. The same study shows that participants with asthma, diabetes, cardiovascular symptoms, or any combination of these diseases had greater odds of experiencing stress, anxiety, and depression than healthy individuals [20].



Graph 1. Mean scores on the DASS-21 scales among the healthy and chronically ill
Grafikon 1. *Prosečne vrednosti na DASS–21 skali kod zdravih i ispitanika sa hroničnim bolestima*

Legend: M - Mean; p - Statistical significance; Note: Independent Samples T-Test was performed
Legenda: M – aritmetička sredina; p – statistička značajnost; Napomena: primenjen je T-test za velike nezavisne uzorke

Table 4. Linear regression models of the association between health status and anxiety
Tabela 4. *Linearni regresioni modeli povezanosti zdravstvenog statusa ispitanika i anksioznosti*

	Chronic illnesses model/*Model sa hroničnim bolestima*	Basic model *Osnovni model*	Full model *Kompletan model*
Presence of chronic diseases (ref.: no diseases)/*Prisustvo hroničnih bolesti (ref.: bez prisustva bolesti)*			
Yes/*Da*	0.17 (0.73 - 2.29) **	0.18 (0.68 - 2.44) **	0.19 (0.80 - 2.55) **
Gender (ref.: males)/*Pol (ref.: muški pol)*			
Females/*Žene*		0.18 (0.83 - 2.36) **	0.16 (0.63 - 2.17) **
Age/*Starost*		-0.06 (-0.04 - 0.01)	-0.14 (-0.08 - 0.00)
Education (ref.: ≥12 years)/*Obrazovanje (ref.: ≥12 godina)*			
≤ 8			0.02 (-1.13 - 1.84)
8 – 12			-0.04 (-1.23 - 0.44)
Employment status (ref.: student)/*Radni status (ref.: student)*			
Unemployed/*Nezaposlen/a*			0.27 (1.49 - 5.42) **
Employed in private sector *Zaposlen/a u privatnom sektoru*			0.00 (-1.93 - 2.08)
Employed in public sector *Zaposlen/a u državnom sektoru*			0.02 (-1.79 - 2.40)
Farmer/homemaker/*Poljoprivrednik/domaćica*			0.03 (-1.66 - 2.90)
Retired/*Penzioner/ka*			0.15 (-0.87 - 4.00)
Family material status (ref.: above average)/*Imovinsko stanje porodice (ref.: iznad proseka)*			
Below average/*Ispod proseka*			-0.03 (-1.84 - 1.74)
Average/*Prosečno*			0.01 (-0.82 - 1.01)
Marital status (ref.: widowed)/*Bračni status (ref.: udovac/udovica)*			
Married/*Oženjen/udata*			-0.04 (-1.70 - 0.94)
Single/*Neoženjen/neudata*			-0.11 (-2.93 - 0.48)
Divorced/*Razveden/a*			-0.05 (-2.06 - 1.89)
Type of current residence (ref.: rural)/*Mesto stanovanja (ref.: manje mesto/selo)*			
Urban/*Grad*			0.07 (-0.70 - 0.82)

Note: Values represent Beta coefficients with corresponding 95% confidence intervals; *$p < 0.05$; **$p < 0.01$
*Napomena: Vrednosti predstavljaju beta koeficijente sa 95% intervalom poverenja; *$p < 0.05$; **$p < 0.01$*

Table 5. Linear regression models of the association between health status and stress
Tabela 5. *Linearni regresioni modeli povezanosti zdravstvenog statusa ispitanika i stresa*

	Chronic illnesses model/*Model sa hroničnim bolestima*	Basic model *Osnovni model*	Full model *Kompletan model*
Presence of chronic diseases (ref.: no diseases)/*Prisustvo hroničnih bolesti (ref.: bez prisustva bolesti)*			
Yes/*Da*	0.11 (0.26 - 2.23)*	0.10 (0.24 - 2.20)*	0.09 (0.01 - 2.13)*
Gender (ref.: males)/*Pol (ref.: muški pol)*			
Females/*Žene*		0.35 (2.88 - 4.73) **	0.34 (2.77 - 4.64)**
Age/*Starost*		-0.03 (-0.03 - 0.03)	0.34 (2.77 - 4.64)**
Education (ref.: ≥12 years)/*Obrazovanje (ref.: ≥ 12 godina)*			
≤ 8			0.03 (-1.17 - 2.43)
8 – 12			0.01 (-0.81 - 1.21)
Employment status (ref.: student)/*Radni status (ref.: student)*			
Unemployed/*Nezaposlen/a*			0.15 (0.10 - 4.87)*
Employed in private sector/*Zaposlen/a u privatnom sektoru*			-0.07 (-3.30 - 1.56)
Employed in public sector/*Zaposlen/a u državnom sektoru*			-0.04 (-3.14 - 1.94)
Farmer/homemaker/*Poljoprivrednik/domaćica*			-0.05 (-3.93 - 1.60)
Retired/*Penzioner/ka*			-0.05 (-3.64 - 2.27)
Family material status (ref.: above average)/*Imovinsko stanje porodice (ref.: iznad proseka)*			
Below average/*Ispod proseka*			0.02 (-1.61 - 2.73)
Average/*Prosečno*			0.05 (-0.50 - 1.72)
Marital status (ref.: widowed)/*Bračni status (ref.: udovac/udovica)*			
Married/*Oženjen/udata*			0.12 (-0.25 - 2.96)
Single/*Neoženjen/neudata*			-0.07 (-3.04 - 1.11)
Divorced/*Razveden/a*			0.06 (-0.85 - 3.95)
Type of current residence (ref.: rural)/*Mesto stanovanja (ref.: manje mesto/selo)*			
Urban/*Grad*			0.04 (-0.48 - 1.37)

Note: Values represent Beta coefficients with corresponding 95% confidence intervals; *$p < 0.05$; **$p < 0.01$;
*Napomena: Vrednosti predstavljaju beta koeficijente sa 95% intervalom poverenja; *$p < 0.05$; **$p < 0.01$;*

Conclusion

The present study examined the mental health of chronically ill adults at the time of the coronavirus disease 2019 pandemic in Serbia. The study was conducted approximately five months after the pandemic was pronounced. One of the causes of high mortality risk in the chronically ill population is their undermined mental health. The findings indicate that the existence of a chronic illness is associated with raised levels of depression, anxiety, and stress.

A limiting factor for the scope of this study is the lack of data regarding pre-pandemic levels of depression, anxiety, and stress. Because of this, before-and-after analysis could not be carried out. However, longitudinal studies that may well be conducted would contribute to a clearer understanding of the population's mental health, focusing in particular on the most vulnerable groups.

Considering that our findings suggest that the mental health of the chronically ill during the coronavirus disease 2019 pandemic is damaged, it is necessary to address this issue with timely social and health measures. We believe that this kind of research may contribute to the development of social and health strategies in order to mitigate the psycho-social effects of the pandemic.

References

1. World Health Organization. Noncommunicable diseases [Internet]. 2018 [cited 2020 Sep 3]. Available from: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases.

2. Chen Q, Liang M, Li Y, Guo J, Fei D, Wang L, et al. Mental health care for medical staff in China during the COVID-19 outbreak. Lancet Psychiatry. 2020;7(4):e15-6.

3. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. 2020 [cited 2020 Sep 2]. Available from: https://covid19.who.int/?gclid=Cj0KCQjwy8f6BRC7ARIsAPIXOjijXNgn1KkU4qPxh7J6eFB5SVjM3nhHDDNGWrDyFbJoAYH5Bw_8qBQaAgKPEALw_wcB.

4. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323(13):1239-42.

5. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA. 2020;323(18):1775-6.

6. Wouts L, Oude Voshaar RC, Bremmer MA, Buitelaar JK, Penninx BW, Beekman AT. Cardiac disease, depressive symptoms, and incident stroke in an elderly population. Arch Gen Psychiatry. 2008;65(5):596-602.

7. Gremigni P, Sciarroni L, Pedrini L. Cognitive changes after carotid endarterectomy. Monaldi Arch Chest Dis. 2009;72(2):91-7.

8. Bugarski V, Sakač V, Vodopivec S, Slankamenac P. Povezanost dimenzija ličnosti i depresivnih obeležja kod pacijenata na hemodijalizi. Med Pregl. 2010;63(5-6):305-12.

9. Vučurević M, Vujović M, Stojanović-Tasić M, Marić NP. Frequency and correlates of depression at the primary health care level in Belgrade. Srp Arh Celok Lek. 2020;148(3-4):185-90.

10. Novaković M. Anksioznost kod pacijenata na dijalizi. Med Pregl. 2007;60(9-10):484-8.

11. Stanimirović L. Anksioznost i depresivnost kod bolesnica sa karcinomom grlića materice pre operativnog lečenja. Sestrinska reč. 2017;21(74):21-3.

12. Özdin S, Bayrak Özdin Ş. Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: the importance of gender. Int J Soc Psychiatry. 2020;66(5):504-11.

13. Louvardi M, Pelekasis P, Chrousos G, Darviri C. Mental health in chronic disease patients during the COVID-19 quarantine in Greece. Palliat Support Care. 2020:1-6.

14. Ozamiz-Etxebarria N, Dosil-Santamaria M, Picaza-Gorrochategui M, Idoiaga-Mondragon N. Stress, anxiety, and depression levels in the initial stage of the COVID-19 outbreak in a population sample in the northern Spain. Cad Saude Publica. 2020;36(4):1-9.

15. World Health Organization. Gaining health. The European Strategy for the Prevention and Control of Noncommunicable Diseases [Internet]. 2006 [cited 2020 Jul 26]. Available from: https://www.euro.who.int/en/publications/abstracts/gaining-health.-the-european-strategy-for-the-prevention-and-control-of-noncommunicable-diseases.

16. Jovanović V, Gavrilov-Jerković V, Žuljević D, Brdarić D. Psihometrijska evaluacija Skale depresivnosti, anksioznosti i stresa-21 (DASS-21) na uzorku studenata u Srbiji. Psihologija. 2014;47(1):93-112.

17. Mazza C, Ricci E, Biondi S, Colasanti M, Ferracuti S, Napoli C, et al. A Nationwide survey of psychological distress among Italian people during the COVID-19 pandemic: immediate psychological responses and associated factors. Int J Environ Res Public Health. 2020;17(9):3165.

18. Wang C, Pan R, Wan X, Tan Y, Xu L, McIntyre RS, et al. A longitudinal study on the mental health of general population during the COVID-19 epidemic in China. Brain Behav Immun. 2020;87:40-8.

19. Boričić K. Rezultati istraživanja zdravlja stanovništva Srbije 2013. godina. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2014.

20. Sayeed A, Kundu S, Al Banna MH, Christopher E, Hasan MT, Begum MR, et al. Mental health outcomes of adults with comorbidity and chronic diseases during the COVID-19 pandemic: a matched case-control study [Preprint]. PsyArXiv [Internet] 2020 [cited 2020 Sep 2]. Available from: https://psyarxiv.com/qh6b5/.

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STRESS FACTORS IN PERSONS WITH INTELLECTUAL DISABILITIES IN DIFFERENT TYPES OF HOUSING

FAKTORI STRESA KOD OSOBA SA INTELEKTUALNOM OMETENOŠĆU U RAZLIČITIM TIPOVIMA STANOVANJA

Daniela TAMAŠ[1], Nina BRKIĆ JOVANOVIĆ[2] and Danica VASILJEVIĆ[3]

Summary

Introduction. Type of housing is of great importance for the overall quality of life and general psychosocial well-being of persons with intellectual disabilities, as evidenced by the results of numerous studies showing that people with intellectual disabilities living in institutions have a lower level of life satisfaction compared to those living in the open community. **Material and Methods.** In order to determine the levels of experienced stress related to the type of housing in people with intellectual disabilities, we carried out a study including 122 persons with intellectual disabilities living in different types of housing; in institutions (n = 51), with families (n = 38), and in the supported housing program (n = 33). The Life Stress Inventory was used for the purpose of this research. **Results.** The results showed that people who were included in the supported housing program experienced the least stress, compared to the subjects who lived with their families or in institutions ($p < 0.001$). Stress was the least prevalent in the supported housing program, but the other two groups had similar results. The presence of stress did not differ significantly between subjects living with their families and those living in institutions. **Conclusion.** We can conclude that subjects living in supported housing experienced significantly less stress compared to the other two groups. Different types of housing were associated with different levels of stress. Overall, negative interpersonal relationship was identified as the stress factor that correlated most significantly with other stress factors.

Key words: Intellectual Disability; Stress, Psychological; Housing; Quality of Life; Interpersonal Relations; Risk Factors

Sažetak

Uvod. Tip stanovanja može da bude od velikog značaja za sveukupan kvalitet života i opšte psihosocijalno stanje osoba što potvrđuju rezultati brojnih istraživanja koji pokazuju da ove osobe koje žive u instituciji imaju niži stepen zadovoljstva životom u poređenju sa ispitanicima koji žive u otvorenoj zajednici. **Materijali i metode.** Radi utvrđivanja nivoa doživljenog stresa u odnosu na tip stanovanja osoba sa intelektualnom ometenošću uradili smo istraživanje kojim su obuhvaćene 122 osobe sa intelektualnom ometenošću koje stanuju u različitim tipovima stanovanja: u instituciji (n = 51), sa porodicama (n = 38) i u programu stanovanja uz podršku (n = 33). Za potrebe istraživanja korišćen je *Upitnik za procenu stresnih životnih događaja.* **Rezultati.** Rezultati pokazuju da osobe koje su obuhvaćene programom stanovanja uz podršku u odnosu na ispitanike koji stanuju sa porodicama i u instituciji najmanje ispoljavaju stres ($p < 0,001$). Ispitanici iz programa stanovanja uz podršku pokazuju najmanje izraženo prisustvo stresa, dok ostala dva poduzorka imaju približne rezultate. Rezultati o prisustvu stresa ispitanika koji žive sa porodicama ne razlikuju se značajno od poduzorka ispitanika koji žive u instituciji. **Zaključak.** Možemo da zaključimo da ispitanici koji žive u programu stanovanja uz podršku u statistički značajno manjoj meri ispoljavaju stres u odnosu na ostala dva poduzorka. U različitim tipovima stanovanja faktori stresa su različito zatupljeni. Na opštem uzorku negativni međuljudski odnosi kao faktor stresa najviše statistički značajno korelaju sa ostalim faktorima stresa.

Ključne reči: intelektualna ometenost; stres; stanovanje; kvalitet života; međuljudski odnosi; faktori rizika

Introduction

Previously, it was thought that persons with intellectual disability (ID) have enough intellectual capacity to experience stress that may lead to mental health problems. The bulk of research is focused on the stress of parents and staff working with persons with ID, rather than the stress of persons with ID. Only a few studies have dealt with the impact of environmental factors on the stress levels in persons with ID [1].

Today's researches show that, compared to other populations, persons with ID are at increased risk of stress due to reduced intellectual skills, difficulty in information processing, need for a structured and predictable environment, and repetitive and restricted behaviors [2, 3]. Also, persons with ID are at significantly higher risk of exposure to adverse social impact of environmental factors, and characteristics of intellectual disability, such as insufficiently developed adaptive capabilities, are associated with increased sensitivity to particular forms of psychopathology [4–6]. A small number of studies dealing with the connection between social environmental factors and psychiatric disorders in children with and without ID are due to the fact that the subjective experience of stress in persons with ID is often difficult to assess because

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Abbreviations
ID – intellectual disability
ANOVA – analysis of variance
ANCOVA – univariate analysis of covariance
LSD – least significant difference
ComQol – comprehensive quality of life scale

of the difficulty in communicating and expressing the suffered stress [7]. The way of experiencing stress may be considered as a predictor of many psychopathological symptoms in persons with ID [8]. As an indication of stress, endocrine function, vital signs, temperature, and physical manifestations can be monitored in persons with ID. A wide range of negative effects in their environment may have a negative impact on the general health status of persons with ID [9].

Dealing with stress involves a set of cognitive and behavioural efforts to overcome stressful situations and/or emotions that these situations cause [10]. In persons with ID, the coping skills are insufficiently developed, and as a result, they experience stress more often, more intensely and longer than the general population. Adults with ID are more vulnerable to stressful social interactions, and they are the most intense and most common source of stress, compared to other categories of stressful events [11]. Active coping with stressful situations in the areas of social interaction is less common in persons with ID, avoiding stressful situations is more common [12]. The significance of environmental factors impacting the mental state was reported by Emerson and Hatton, who found that social environmental factors were usually expressed as three categories of psychiatric disorders: conduct disorders, emotional disorders, including anxiety disorder, and hyperkinesis [4]. Scott and Havercamp found that lack of social support was associated with occurrence/development of mental illness in persons with ID [11].

Type of housing can be of great importance for the overall quality of life and general psychosocial conditions of persons with ID, as evidenced by the results of numerous studies showing that persons with ID living in institutions have a lower level of life satisfaction compared to those living in the open community.

Supported housing for the persons with ID represents the favour in the system of social protection which enables them more independent life in the least restrictive surrounding. Adequate accommodation, skilled help and support for more independence and participation of the users in the social community are provided by applying the favour of the supported housing program. Reasons for supported housing for the persons with ID are complex and vary across national political contexts; one common factor is the embrace by advocates of the concept of normalization and the rejection of segregation of persons with ID from the rest of society. Supported housing for the persons with ID should be more than just "a change of address" and coincides with the emergence of a set of assumptions acknowledging that persons are capable of making choices about their own lives, respecting their right to do so, and focusing on individualized support and empowerment [13, 14].

The subjective satisfaction with living conditions of persons with ID is statistically significantly associated with social participation and opportunities for utilization of different social resources [13]. With regard to contacts with the social environment, friendly relationships and activities with friends are closely associated with the subjective feeling of quality of life as confirmed in a research by Duvdevany and Arar [15]. Persons with ID who live in institutions and even those living with their families have an evident deficit in social interactions [16].

Bramston et al. [17] reported that quality of life is multidimensional and affected by social environmental factors and personal interaction with the environment, and may be improved by the possibility of self-determination, finding one's purpose in life and having a sense of belonging. One of the most important domains for the quality of life of the older population is related to family and social networks [18]. According to the results of previous research, which used a methodology based on the opinions of individuals, interrelationships are most important in quality of life of the elderly [19, 20].

Taking into account that stress is the relation of a person and the environment, within which the person perceives that some aspects of the environment represent a threat, loss or challenge to their powers, which causes characteristic changes in the psycho-physiological equilibrium [21], then different types of housing and living conditions also may be potential stressors for persons with ID.

Bramston et al. carried out a study on stress in persons with ID, using the Life Stress Inventory. Based on gathered results, they identified three key stress factors in persons with ID: general concern (support of family, friends and partners, violation of rights, possibilities of choice, coercion and invasion of privacy), negative interpersonal relationships, and lack of personal competence [22].

Material and Methods

The objective of this study was to evaluate the level of stress experienced by persons with ID and to determine the dominant stress factors in relation to the type of housing, as well as to examine the correlation between subjective perception of satisfaction and importance of quality of life and stress. The total number of subjects was 122; 51 (41.8%) lived in institutions, 38 (31.1%) lived with their families, and 33 (27%) were included in the program of supported housing. Data on sex and age were taken from the subjects' files kept by institutions. The $\chi 2$ test was used to test if there were differences between the three sub-samples of subjects with regard to sex. Despite the fact that the three sub-samples differed with regard to sex distribution, the difference was not statistically significant ($\chi 2 = 1.06$, $p = 0.59$).

The average age of the entire sample was 39.95 years. Based on the results of analysis of variance (ANOVA), we found a statistically significant difference in age ($F = 61.00$, $p < 0.001$). The age dif-

ference was particularly pronounced among subjects living in institutions (average age: 49.43 years) and those living with their families (average age: 26.32 years), whereas the average age of subjects living in supported housing was 41.10 years. The post-hoc analysis using the least significant difference (LSD) test confirmed the presence of significant differences between all three subsamples ($p < 0.001$ for each of the three differences).

The data on the medical state of the subjects showed different comorbid conditions. Their diversity was so great that the influence of individual states on the dependent variables in this study could not be controlled. For this reason, the analysis of their impact on dependent variables has not been done, which may be the lack of this study.

We used the Life Stress Inventory [22] and this instrument has not previously been used in the domestic population. For research purposes, the questionnaire was translated.

The questionnaire was designed so that the respondents could assess certain potentially stressful events and consists of two parts: a questionnaire for assessment of stressful life events - self-assessment (Life Stress Inventory, self-report), which represents the original version of the questionnaire and is intended for persons with ID, and a questionnaire for the assessment of stressful life events - an objective scale (Life Stress Information), which is intended for parents and caregivers, modified in such a way that the same questions were answered by another person [22]. Both questionnaires consisted of 30 questions related to life events that most often lead to a state of stress in persons with ID. They included Likert-type questions, with responses ranging from 0 to 2 (no – 0; sometimes – 1; yes – 2]. The higher values indicated a higher level of stress among the subjects' sum of results and represented the total number of life events that have had a recent impact on their lives. The reliability of the Life Stress Information, calculated by the authors, based on the research is adequate; Cronbach's alpha - 0.70, whereas the Life Stress Inventory, a self-report, has even greater reliability (Cronbach's alpha - 0.80) [23].

On the basis of factor analysis, which was done by the authors of the instrument, results can be categorized into three subscales: 1. General concern; 2. Negative interpersonal relationships; and 3. Sense of lack of personal competence.

To measure the quality of life, we used the Comprehensive Quality of Life Scale (ComQol–I5, Fifth Edition), designed for persons who have ID or other form of cognitive impairment [24]. The ComQol is a multidimensional scale; it defines life quality in terms of seven domains: material well-being, health, productivity, intimacy, safety, place in the community, and emotional well-being, which together include all quality of life components. The scale is also multi-axial; it has two forms and the first is in the separate measurement of objective and subjective components. Each domain is separately rated in terms of its importance to the individual as well as perceived satisfaction.

The data resulting from our research revealed a high reliability of the instrument, as shown by the results of Cronbach's alpha coefficient, ranging from 0.79 to 0.97; 0.79 for Stress - objective; and 0.83 for Stress – subjective.

The quality of life was measured using ComQol–I5, and the results ranging from 0.79 – 0.87 show good relialability (subjective perception of importance 0.82, subjective perception of satisfaction 0.79, and the expert's evaluation – importance 0.86; satisfaction 0.87). The study was approved by directors and coordinators of institutions and organizations where the study was carried out, who were informed about the purpose and course of the study. The questionnaire was applied by the professional staff working in institutions and organizations providing support to the subjects, who by the nature of their work were in contact with the subjects and were familiar with their condition and individual characteristics. We are fully aware that one of the disadvantages that may impact the results could be bias point of view of professional staff while filling in the questionnaire. Since the subjects have no verbal abilities and their cognitive status is not well preserved, it was necessary to incorporate professional staff familiar with the examinees. The part of the instrument that tested the subjective experience of subjects (Life Stress Inventory, self-report) was performed with support of the professional staff.

Study limitations

Apart from the fact that we could not control the diverse comorbid conditions, already mentioned above when describing exclusion criteria of the study, we are fully aware that one of the disadvantages that may impact the results of the research could be the point of view of professional staff while filling in the questionnaire. Since respondents with ID have a deficit in communication skills and their cognitive status is not well preserved, it was necessary to include the professional staffs that were familiar with the examinees.

For the statistical analysis, we used Statistical Package for the Social Sciences (SPSS) version 20. Univariate analysis of covariance (ANCOVA) and LSD post-hock test were used to identify intergroup differences. The correlations were tested using Pearson's correlation coefficient.

Results

Table 1 shows that age had no statistically significant effects on the differences in the level of stress in the three subsamples of subjects, whereas the type of housing had a statistically significant effect on this indicator ($p < 0.01$).

The results shown in **Table 2** indicate that there was a statistically significant difference between subjects from supported housing compared to the other two groups ($p < 0.001$).

Table 3 shows that the analysis of objective and subjective responses yielded a statistically significant difference between the three sub-samples with

Table 1. Effects of age and type of housing on indicators of stress level (ANCOVA)[1]
Tabela 1. *Efekti starosti i tipa stanovanja na pokazatelje nivoa stresa (ANCOVA)[1]*

Effects/*Efekti*	Dependent variables/*Zavisne varijable*	SS/*SS*	df/*df*	MS/*MS*	F/*F*	p/*p*	P.Eta2/*P.Eta2*
Age *Starost*	STRESS subjective/*STRES subjektivni*	16.82	1	16.82	0.25	0.62	0.002
	STRESS objective/*STRES objektivni*	5.39	1	5.39	0.10	0.76	0.001
Type of housing *Tip stanovanja*	STRESS subjective/*STRES subjektivni*	1373.67	2	686.84	10.21	0.00	0.165
	STRESS objective/*STRES objektivni*	809.02	2	404.51	7.21	0.00	0.123

[1]The following tags are used in tables to represent different stress factors: • Objective assessment: s1o - general concern; s2o - negative interpersonal relationships; s3o - sense of lack of competence, • Subjective assessment: s1s - general concern; s2s - negative interpersonal relationships; s3s - a sense of lack of competence
[1]Tabele koriste sledeće oznake za predstavljanje različitih faktora stresa: • Objektivna procena: s1o – opšta zabrinutost; s2o – negativni međuljudski odnosi; s3o – osećaj nedostatka kompetencije, • Subjektivna procena: s1s – opšta zabrinutost; s2s – negativni međuljudski odnosi; s3s – osećaj nedostatka kompetentnosti

Table 2. Differences in stress levels between the three sub-samples
Tabela 2. *Razlike u nivou stresa između tri poduzorka ispitanika*

		AS/*AS*	SD/*SD*	F/*F*	df/*df*	p/*p*
STRESS subjective *STRES subjektivni* Significant diff: *Značajne razlike:* 1 - 3, 2 - 3	Institution/*Institucija*	23.37	9.03	10.432	2. 107	0.000
	Family/*Porodica*	24.46	8.54			
	Supported housing/*Stanovanje uz podršku*	15.33	4.88			
STRESS objective *STRES objektivni* Significant diff: *Značajne razlike:* 1 - 3, 2 - 3	Institution/*Institucija*	25.78	7.75	10.511	2. 116	0.000
	Family/*Porodica*	27.00	8.01			
	Supported housing/*Stanovanje uz podršku*	19.41	5.87			

Table 3. Presence of different stressors in the three sub-samples and comparison of subjective and objective results
Tabela 3*. Prisutnost različitih stresora u tri poduzorka i poređenje subjektivnih i objektivnih rezultata*

		SS - sum of squares of mean deviations/***SS -*** *suma kvadrata odstupanja od srednje vrednosti*	df df	MS – variance *MS – varijansa*	F *F*	p *p*
s1o	Between sub-samples/*Između tri poduzorka*	639.540	2	319.770	18.834	0.000
	Within sub-sample/*U okviru poduzorka*	2020.436	119	16.978		
	Total/*Total*	2659.975	121			
s2o	Between sub-samples/*Između tri poduzorka*	3.569	2	1.784	0.225	0.799
	Within sub-sample/*U okviru poduzorka*	943.816	119	7.931		
	Total/*Total*	947.385	121			
s3o	Between sub-samples/*Između tri poduzorka*	62.907	2	31.454	8.923	0.000
	Within sub-sample/*U okviru poduzorka*	419.495	119	3.525		
	Total/*Total*	482.402	121			
s1s	Between sub-samples/*Između tri poduzorka*	419.712	2	209.856	15.807	0.000
	Within sub-sample/*U okviru poduzorka*	1566.536	118	13.276		
	Total/*Total*	1986.248	120			
s2s	Between sub-samples/*Između tri poduzorka*	63.458	2	31.729	2.828	0.063
	Within sub-sample/*U okviru poduzorka*	1335.009	119	11.219		
	Total/*Total*	1398.467	121			
s3s	Between sub-samples/*Između tri poduzorka*	51.417	2	25.709	6.851	0.002
	Within sub-sample/*U okviru poduzorka*	446.583	119	3.753		
	Total/*Total*	498.000	121			

regard to the dominating stress factors: general concern and sense of lack of competence.

The post-hoc analysis, using the LSD test, showed the differences between the impacts of three different stressors on the presence of stress in relation to the type of housing, based on the answers on the objective and subjective subscales (**Table 4** and **Graph 1**). The sub-samples of subjects are represented by numbers (1 - living in an institution, 2 - living with their families, and 3 – supported housing).

At the level of the whole sample, **Table 5** shows the correlation between subjective perception of

Table 4. Presence of stressors in the three sub-samples; Post Hoc Tests LSD
Tabela 4*. Prisutnost faktora stresa u sva tri poduzorka ispitanika; Post Hoc Tests LSD*

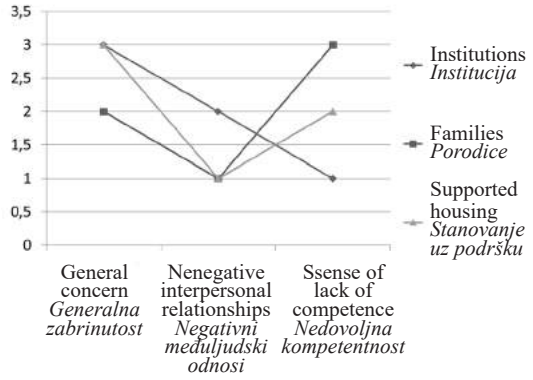
Dependent variables *Zavisne varijable*	(I) group *(I) grupa*	(J) group *(J) grupa*	Mean difference (I - J) *Razlike između srednjih vrednosti (I - J)*	Stdandard deviation *Standardna greška*	p *p*	95% Confidence interval *95% Interval pouzdanosti*	
						Lower limit *Donja granica*	Upper limit *Gornja granica*
s1o	1	2	1.13364	0.88301	0.202	-0.6148	2.8821
		3	-4.55615*	0.92055	0.000	-6.3789	-2.7334
	2	1	-1.13364	0.88301	0.202	-2.8821	0.6148
		3	-5.68979*	0.98046	0.000	-7.6312	-3.7484
	3	1	4.55615*	0.92055	0.000	2.7334	6.3789
		2	5.68979*	0.98046	0.000	3.7484	7.6312
s2o	1	2	0.31992	0.60352	0.597	-0.8751	1.5149
		3	0.37255	0.62917	0.555	-0.8733	1.6184
	2	1	-0.31992	0.60352	0.597	-1.5149	0.8751
		3	0.05263	0.67012	0.938	-1.2743	1.3795
	3	1	-0.37255	0.62917	0.555	-1.6184	0.8733
		2	-0.05263	0.67012	0.938	-1.3795	1.2743
s3o	1	2	-1.68989*	0.40235	0.000	-2.4866	-0.8932
		3	-0.89483*	0.41946	0.035	-1.7254	-0.0643
	2	1	1.68989*	0.40235	0.000	0.8932	2.4866
		3	0.79506	0.44676	0.078	-0.0896	1.6797
	3	1	0.89483*	0.41946	0.035	0.0643	1.7254
		2	-0.79506	0.44676	0.078	-1.6797	0.0896
s1s	1	2	0.02120	0.78684	0.979	-1.5370	1.5793
		3	-4.17291*	0.81400	0.000	-5.7849	-2.5610
	2	1	-0.02120	0.78684	0.979	-1.5793	1.5370
		3	-4.19410*	0.87241	0.000	-5.9217	-2.4665
	3	1	4.17291*	0.81400	0.000	2.5610	5.7849
		2	4.19410*	0.87241	0.000	2.4665	5.9217
s2s	1	2	-0.13725	0.71777	0.849	-1.5585	1.2840
		3	1.55971*	0.74828	0.039	0.0780	3.0414
	2	1	0.13725	0.71777	0.849	-1.2840	1.5585
		3	1.69697*	0.79698	0.035	0.1189	3.2751
	3	1	-1.55971*	0.74828	0.039	-3.0414	-0.0780
		2	-1.69697*	0.79698	0.035	-3.2751	-0.1189
s3s	1	2	-1.49639*	0.41514	0.000	-2.3184	-0.6744
		3	-0.30660	0.43279	0.480	-1.1636	0.5504
	2	1	1.49639*	0.41514	0.000	0.6744	2.3184
		3	1.18979*	0.46095	0.011	0.2771	2.1025
	3	1	0.30660	0.43279	0.480	-0.5504	1.1636
		2	-1.18979*	0.46095	0.011	-2.1025	-0.2771

* Mean is significant at the level 0.05/* *Srednja vrednost je značajna na nivou 0,05*

Table 5. Correlation between subjective perception of satisfaction with the quality of life and importance of quality of life and stress among the examinees; correlation between nonbiased observations of the staff on perception of satisfaction of examinees and giving importance to quality of life and stress; at the level of whole sample
Tabela 5. *Korelacije između subjektivne percepcije zadovoljstva kvalitetom života i važnosti kvaliteta života i stresa ispitanika; korelacija između objektivnih zapažanja osoblja o percepciji zadovoljstva ispitanika, njihovom pridavanju važnosti kvalitetu života i stresu ispitanika; na nivou celog uzorka*

	Satisfaction/*Zadovoljstvo*		Importance/*Važnost*	
	Subjective scale *Subjektivna skala*	Objective scale *Objektivna skala*	Subjective scale *Subjektivna skala*	Objective scale *Objektivna skala*
Stress/Subjective/*Stres/Subjektivno*	-0.422**	-0.389**	-0.177	-0.299**
Stress/Objective/*Stres/Objektivno*	-0.244**	-0.353**	-0.052	-0.224*

Note: ** p < 0.01, * p < 0.05



Graph 1. Distribution of stress factors in different types of housing
Grafikon 1*. Zastupljenost faktora stresa u različitim tipovima stanovanja*

satisfaction with the quality of life and importance of quality of life and stress among the examinees and correlation between nonbiased observations of the employers on the examinees' perception of satisfaction and giving importance to quality of life and stress.

Discussion

The results of our study show that the type of housing has a significant effect on the differences in the level of stress in the three sub-samples of subjects. Subjects from the supported housing program had the least stress, while the other two subsamples had similar results. Our findings suggest that the presence of stress differs depending on the type of housing of persons with ID, which coincides with the results of Hartley and Mac Lane [25].

In **institutions**, stress occurred most commonly due to general concerns which may have been caused by a reduced possibility of choice, invasion of privacy, inadequate relationship with the environment, inadequate approach and lack of individualization in work, leading to reduced fulfilment of personal interests, affinities and needs. The fact that the stress caused by the lack of sense of competence in these subjects was the lowest, may be explained by the fact that in their routines and not very demanding environment they did not feel so incompetent.

On the other hand, persons who lived in their **families** showed the greatest stress related to the lack of competence; a possible explanation is overprotective attitude of their parents, coping with new situations and experiences, but also with reduced choice and decision-making. The least prevalent was stress due to negative interpersonal relationships, which may be explained by a stronger support in their environment.

In **supported housing,** general concern was the most common cause of stress. It is possible that this is because in an open community persons who live in supported housing often face new situations that deviate from the routine, they lack understanding of social norms and perceive some situations as a violation of their rights and fostering of their autonomy as a lack of support. Stress due to negative interpersonal relationships was the least prevalent stress factor, maybe because of expert supervision of small groups which enables quick and efficient interventions. The subjects rely on the support of the staff similarly as those living in family rely on the support of their family members.

Based on the results of our research, we came to the conclusion that type of housing has a significant influence on persons with ID regarding higher quality life and realizing the importance of achieving that goal in certain areas of development. Subjects who are included in the supported housing are fairly included into social interactions with other persons as well as in some social groups, which is not the case in the other two groups. Our results showed that, regardless of the type of housing, there are statistically significant and negative correlations when assessing satisfaction with quality of life and subjective perception of stress and objective estimate of stress by caregivers.

Persons with ID who are included in the supported housing are more satisfied with quality of life in certain aspects, more than other two subcategories of the sample. However, regardless of the living conditions and the type of housing, respondents are not giving importance to the material aspect of their time spent there. Something that is statistically significant and also correlated with the type of housing is the perception of importance and satisfaction with quality of life which coincides with client's opportunity to carry out certain activities with persons who do not share the same living environment and are not socially affiliated with them.

Using factor analysis of the Life Stress Inventory, the authors of the instrument obtained indications on the type of stressors that the study population was susceptible to, i.e. three stress factors: general concern, negative interpersonal relationships and sense of lack of competence. Based on their findings, negative interpersonal relationships was identified as the most pronounced stress factor in persons with ID [22]. On the basis of this division, we evaluated the presence of these stressors in each sub-sample and compared subjective and objective results in three sub-samples, using the one-factor analysis of variance (ANOVA). According to our results, the dominating stressor that statistically significantly differed was the type of housing.

On the basis of objective responses, we obtained statistically significant results which indicated that persons who lived in institutions and in families were under greater stress when it comes to general concerns than subjects who lived in supported housing ($p < 0.001$). The stress that resulted from negative interpersonal relationships was significantly greater in subjects who lived in supported housing compared with those who lived in institutions ($p = 0.039$) and those who lived with their families ($p = 0.035$). The pronounced stress due to negative interpersonal relationships in persons with ID who

lived in supported housing may be a result of their frequent facing inadequate and discriminatory attitudes of the environment in everyday life situations. Social support is a component that is usually associated with most models of stress and can often be seen as an agent or factor that helps to reduce the impact of severe stressors [26–28]. Persons with ID often depend on their family members and on support of professional staff in various aspects of life, which is the reason that social support is a key component of stress. According to the research, persons with ID living in institutions often rely on staff to manage stress, while those who live with their families have the care and support of their family members [25, 27]. A sense of lack of competence as a cause of stress, according to the subjective responses, was the least prevalent among subjects who lived with their families [13, 29]. There was a statistically significant difference in terms of subjective experience of stress due to sense of lack of competence between subjects living with their families and those living in institutions ($p < 0.001$) and supported housing ($p = 0.011$).

Based on the results of our study, we came to the conclusion that the type of housing has a significant influence on persons with ID; they have a higher quality life and realize the importance of achieving some goals in certain areas of development. Subjects who are included in the supported housing have pretty good social interactions with other persons as well as some social groups, but it is not the case in the other two groups. The results of our and similar studies on this topic showed that, regardless of the type of housing, there are statistically significant and negative correlations between satisfaction with quality of life, subjective perception of stress, and objective presence of stress, when assessed by caregivers [30].

Conclusion

Based on the data obtained in this study, we can conclude that stress is associated with the type of residence; persons who live in supported housing show statistically significantly less stress compared to subjects who live with their families or in institutions.

There are different stress factors in different types of housing. In the overall sample, regardless of the type of housing, the most significant stress factor was caused by negative interpersonal relations in correlation with other stress factors.

Analysis of the prevalence of stress factors in different types of housing is important because it provides a possibility for preventive actions, which would include multi-sector work with persons with intellectual disabilities and their families, as well as mental-hygiene activities in the environment and better training of professional staff in the field of identification of problems and involvement in their solution. Approaches to working with persons with intellectual disabilities, among others, should be focused on reducing their exposure to adverse social conditions and strengthen mechanisms to cope with stress and to overcome it. It is also necessary to develop approaches focused on the family in terms of empowering parents to provide necessary support to persons with intellectual disabilities, but to overcome double and environmental conflicts and stressful family situations.

Future research should focus on the relationship and mutual influence between intellectual disability, social-environmental, psychological and biological factors and their impact on the manifestation of psychiatric disorders and behavioral disorders in persons with intellectual disabilities. Also, research into relationships between social environmental factors and persons with intellectual disabilities could give clearer insights into the nature of stress caused by interpersonal relationships.

References

1. Vrijmoeth C, Monbaliu E, Lagast E, Prinzie P. Behavioral problems in children with motor and intellectual disabilities: prevalence and associations with maladaptive personality and marital relationship. Res Dev Disabil. 2012;33(4):1027-38.

2. Bramston P, Cummins RA. Stress and the move into community accommodation. J Intellect Dev Disabil. 1998;23(4):295-308.

3. Bramston P, Fogarty G, Cummins RA. The nature of stressors reported by people with an intellectual disability. J Appl Res Intellect Disabil. 1999;12(1):1-10.

4. Emerson E, Hatton C. Contribution of socioeconomic position to health inequalities of British children and adolescents with intellectual disabilities. Am J Ment Retard. 2007;112(2):140-50.

5. Schepens HR, Van Puyenbroeck J, Maes B. "One does not forget, it all comes back". Elderly people with intellectual disability review adversities and stress-protection in their lives. Qual Ageing Older Adults. 2019;20(4):190-205.

6. Scott HM. The stress-mental health relationship: social support and physical activity as moderators in adults with intellectual disabilities [thesis]. Columbus, OH: Ohio State University; 2012.

7. Shpigelman CN. Leveraging social capital of individuals with intellectual disabilities through participation on Facebook. J Appl Res Intellect Disabil. 2018;31(1):e79-91.

8. Hulbert-Williams L, Hastings R, Owen DM, Burns L, Day J, Mulligan J, et al. Exposure to life events as a risk factor for psychological problems in adults with intellectual disabilities: a longitudinal design. J Intellect Disabil Res. 2014;58(1):48-60.

9. Emerson E. Commentary: childhood exposure to environmental adversity and the well-being of people with intellectual disabilities. J Intellect Disabil Res. 2013;57(7):589-600.

10. Gardner W. Behavior modification in mental retardation: the education and rehabilitation of the mentally retarded adolescent and adult. Abingdon: Routledge; 2017.

11. Scott HM, Havercamp SM. Mental health for people with intellectual disability: the impact of stress and social support. Am J Intellect Dev Disabil. 2014;119(6):552-64.

12. Scott HM. Social support, physical activity and stress as determinants of well-being in adults with intellectual disability [dissertation]. Columbus, OH: Ohio State University; 2016.

13. Bigby C, Bould E, Beadle-Brown J. Conundrums of supported living: the experiences of people with intellectual disability. J Intellect Dev Disabil. 2017;42(4):309-19.

14. Wong YI, Huangfu Y, Hadley T. Place and community inclusion: locational patterns of supportive housing for people

with intellectual disability and people with psychiatric disorders. Res Dev Disabil. 2018;83:108-19.

15. Duvdevany I, Arar E. Leisure activities, friendships, and quality of life of persons with intellectual disability: foster homes vs. community residential settings. Int J Rehabil Res. 2004;27(4):289-96.

16. Durbin A, Isaacs B, Mauer-Vakil D, Connelly J, Steer L, Roy S, et al. Intellectual disability and homelessness: a synthesis of the literature and discussion of how supportive housing can support wellness for people with intellectual disability. Curr Dev Disord Rep. 2018;5(3):125-31.

17. Bramston P, Chipuer H, Pretty G. Conceptual principles of quality of life: an empirical exploration. J Intellect Disabil Res. 2005;49(Pt 10):728-33.

18. Rojo-Pérez F, Fernández-Mayoralas G, Forjaz MJ, Prieto-Flores ME, Martínez-Martín P. Residential environment and health conditions among older-adults in community-dwelling in Spain: what influences quality of life? In: Environmental gerontology in Europe and Latin America. Cham: Springer International Publishing; 2016. p. 149-74.

19. Bigby C, Beadle-Brown J. Improving quality of life outcomes in supported accommodation for people with intellectual disability: what makes a difference? J Appl Res Intellect Disabil. 2018;31(2):e182-200.

20. Puciato D, Borysiuk Z, Rozpara M. Quality of life and physical activity in an older working-age population. Clin Interv Aging. 2017;12:1627-34.

21. Biggs A, Brough P, Drummond S. Lazarus and Folkman's psychological stress and coping theory. In: Cooper CL. Handbook of stress and health: a guide to research and practice. Chichester, UK: John Wiley & Sons; 2017. p. 349-64.

22. Fogarty GJ, Bramston P, Cummins RA. Validation of the Lifestress Inventory for people with a mild intellectual disability. Res Dev Disabil. 1997;18(6):435-56.

23. Lunsky Y, Bramston P. A preliminary study of perceived stress in adults with intellectual disabilities according to self-report and informant ratings. J Intellect Dev Disabil. 2006;31(1):20-7.

24. Cummins RA. Comprehensive quality of life scale – intellectual disability. 3rd ed. Melbourne, Victoria: Psychology Research Centre, Deakin University; 1992.

25. Hartley SL, MacLean WE. Coping strategies of adults with mild intellectual disability for stressful social interactions. J Ment Health Res Intellect Disabil. 2008;1(2):109-27.

26. Cox NC, Hill AP. Trait perfectionism and attitudes towards people with disabilities. Pers Individ Dif. 2018;122:184-9.

27. Scott HM, Havercamp SM. Comparisons of self and proxy report on health-related factors in people with intellectual disability. J Appl Res Intellect Disabil. 2018;31(5):927-36.

28. Tamaš D, Glumbić N, Golubović Š. Correlation between aggressive behaviour and stress in people with intellectual disability in relation to the type of housing. Journal of Special Education and Rehabilitation. 2016;17(3-4):46-61.

29. Crnic KA, Neece CL, McIntyre LL, Blacher J, Baker BL. Intellectual disability and developmental risk: promoting intervention to improve child and family well-being. Child Dev. 2017;88(2):436-45.

30. Krstić T, Oros M. Coping with stress and adaptation in mothers of children with cerebral palsy. Med Pregl. 2012;65(9-10):373-7.

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ASSESSMENT OF MACROPROLACTINEMIA BY THE POLYETHYLENE GLYCOL PRECIPITATION METHOD

ODREĐIVANJE MAKROPROLAKTINEMIJE PRIMENOM POLIETILEN-GLIKOL METODOLOGIJE

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Summary

Introduction. Macroprolactinemia is one of the common causes of hyperprolactinemia, especially in cases where standard routine commercial immunometric assays are used for prolactin level measurement. Two forms of prolactin, inactive dimeric prolactin (50 - 60 kDa) and a low-activity tetramer (molecular weight above 150 kDa), contribute to this condition. The relatively low incidence of symptoms in macroprolactinemia patients has necessitated a relatively simple method to detect large, biologically inactive prolactin molecules, such as polyethylene glycol precipitation method. The aim of this study was to compare the precipitation method using polyethylene glycol dissolved in phosphate buffered saline in relation to polyethylene glycol dissolved in water. **Material and Methods.** This study included 82 patients who visited the Center of Laboratory Medicine, Clinical center of Vojvodina, to determine serum prolactin levels. The obtained serum samples were divided into two aliquots. The first aliquot was frozen and stored at - 20ºC and the other was immediately analyzed. Aqueous solution was added to one aliquot and polyethylene glycol Merck 6000 to the other. All serum and supernatant samples were analyzed using the automated Abbott Architect i2000sr immunoassay. Recovery values were calculated as the ratio of double values of free prolactin and total prolactin concentration, expressed as a percentage. **Results.** The prolactin concentrations and calculated recovery values were lowest in fresh supernatant prolactin treated with phosphate buffered saline. Statistical analysis showed no significant difference in the calculated recovery values obtained by precipitation of fresh and frozen sera using polyethylene glycol dissolved in phosphate buffered saline ($p = 0.893$). **Conclusion.** Precipitation using polyethylene glycol dissolved in phosphate buffered saline is a more reliable method for laboratory detection of macroprolactinemia compared to polyethylene glycol dissolved in water.

Key words: Hyperprolactinemia; Prolactin; Polyethylene Glycols; Serum; Diagnosis; Clinical Laboratory Techniques; Immunoprecipitation

Sažetak

Uvod. Makroprolaktinemija je jedan od čestih uzroka hiperprolaktinemije u slučajevima kada se za rutinsko određivanje prolaktina koriste standardni, komercijalni, imunometrijski pribori. Ovakvom stanju doprinose dve forme prolaktina, neaktivan dimer (50–60 kDa) i niskoaktivni tetramer (molekulske mase iznad 150 kDa). Relativno mala učestalost kliničkih simtoma u okviru makroprolaktinemije nametnula je potrebu za jednostavnom metodom kojom se detektuju veliki, biološki neaktivni molekuli prolaktina, kao što je precipitaciona metoda polietilenglikolom. Cilj ove studije bio je upoređivanje metode precipitacije pomoću polietilen-glikol metodologije rastvorenog u fosfatnom puferu u odnosu na polietilen-glikol pripremljen sa vodom. **Materijal i metode.** Ovom studijom obuhvaćena su 82 pacijenta, koji su se tokom godinu dana javljali u Centar za laboratorijsku medicinu Kliničkog centra Vojvodine sa zahtevom za laboratorijskim određivanjem koncentracije prolaktina. Dobijeni serumi su razlivani u dva alikvota. Prvi alikvot je zamrzavan i čuvan na temperaturi -20˚ C, a drugi je odmah analiziran. Oba alikvota, svež i zamrzavan tretirani su odgovarajućim vodenim, odnosno fosfatnim rastvorima polietilen-glikola *Merch* 6000. Uzorci su potom analizirani na automatizovanom sistemu *Abbott Architect i2000sr*. *Recovery* vrednosti (Re%) izračunate su kao odnos dvostruke vrednosti slobodnog i ukupnog prolaktina izraženog u procentima. **Rezultati.** Koncentracije prolaktina i izračunate Re% vrednosti najniže su u svežem supernatantu tretiranim fosfatnim rastvorom polietilenglikola. Statističkom obradom nije utvrđena značajna razlika u izračunatim Re% vrednostima dobijenim precipitacijom svežih i zamrzavanih seruma pomoću polietilenglikola rastvorenog u fosfatnom puferu ($p = 0,893$). **Zaključak.** Precipitacija pomoću polietilenglikola rastvorenog u fosfatnom puferu predstavlja pouzdaniju metodu za laboratorijsko otkrivanje makroprolaktinemije, u odnosu na polietilenglikol pripreman s vodom.

Ključne reči: hiperprolaktinemija; prolaktin; polietilen glikol; serum; dijagnostika; kliničko laboratorijske tehnike; imunoprecipitacija

Introduction

Human prolactin (PRL) is a hormone produced by lactotroph cells of the adenohypophysis and its main role is stimulation of lactation. Also, it plays a role in metabolic processes, angiogenesis, regulation of immune system and osmoregulation [1]. An increased secretion of PRL by lactotroph cells leads to hyperprolactinemia which can appear in some physiological and pathological conditions. A certain

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Abbreviations

PRL – prolactin
PEG – polyethylene glycol
PBS – phosphate buffered saline
Re % – recovery value percentage
SD – standard deviation
CV % – percentage of the coefficient of variation

number of hyperprolactinemias are designated as idiopathic, and one of the most common idiopathic hyperprolactinemia is macroprolactinemia. According to the literature, the prevalence of macroprolactinemia in hyperprolactinemic populations varies between 15–30% [2].

Today, the term macroprolactinemia is used for formation of macroaggregates containing several monomer units of PRL. It is believed that macroprolactinemia is caused by PRL antigenicity which leads to the formation of anti-prolactin antibodies that bind to PRL and lead to the formation of large macroprolactin molecules [3]. However, the exact pathogenesis of these antibodies is unknown. The presence of anti-prolactin antibodies reduces PRL bioactivity and slows its clearance. Large macroprolactin molecules cannot pass through the capillary endothelium and reach the target tissues to demonstrate their function and also cannot reach hypothalamic receptors and act as a negative feedback, which together leads to hyperprolactinemia. However, the symptoms of hyperprolactinemia are commonly absent because of low bioactivity of the macroprolactin molecules, as well as because antiprolactin antibodies compete with the monomer PRL molecules for the receptor sites in target tissues, thereby reducing their biological effect [4, 5].

In all cases where PRL macroaggregates are formed, hyperprolactinemia is detected by commercial immunometric assays, because specific antibodies in analytical systems are directed to the corresponding epitopes on the PRL molecule. It is clear that these diagnostic tests also detect total concentration of PRL molecules in cases of macroprolactinemia, where hyperprolactinemia is not accompanied by distinct clinical symptoms or if these symptoms are very discrete.

It can be said that due to the limitations of laboratory diagnostics, macroprolactinemia shows discrepancy between laboratory findings and clinical presentation, which can lead to misdiagnosis or to numerous and often very expensive diagnostic tests [6]. In spite of discrete clinical symptoms, it is necessary to provide a relatively simple, inexpensive and, at the same time, reliable method for detection of large, biologically inactive PRL molecules, thereby avoiding the use of expensive, unnecessary hormonal, radiological and other morpho-functional diagnostic procedures.

Although gel filtration chromatography (GFC) is considered to be the gold standard for detection of macroprolactin molecules, polyethilene glycol (PEG) precipitation method is a simple, inexpensive screening test for macroprolactinemia. It is based on the principle of reducing large macroprolactin molecules by previously prepared PEG solution dissolved in water or in phosphate buffered saline (PBS) and subsequent determination of the PRL concentration in the supernatant.

The aim of the study was to compare the precipitation method using PEG dissolved in PBS in relation to the PEG dissolved in water in order to diagnose macroprolactinemia in everyday laboratory practice.

Material and Methods

This study included 82 patients, 9 men and 73 women, who visited the Center of Laboratory Medicine, Clinical Center of Vojvodina and requested determination of PRL concentration. The study included PRL levels within the reference range, as well as higher and lower levels.

A 5 ml blood was sampled into standard gel separator primary sampling tubes by puncture of the cubital vein. After centrifugation at 4000 rpm for 10 minutes, serum samples were poured into two aliquots. The first aliquot was frozen and stored at - 20°C and the second aliquot was immediately analyzed. Both aliquots, fresh and frozen, were treated with appropriate, aqueous or PBS solutions of PEG. Thereafter, the samples were analyzed using automated Architect i2000sr, Abbott Labs, USA. According to the manufacturer's recommendations; the reference values for serum PRL were: 5.18–26.53 ng/ml for women and 3.46–19.40 ng/ml for men.

The method of precipitation with PEG 6000 Merck was used. Patient serum samples were treated with 25% PEG solution. Two types of 25% PEG solutions were made: aqueous (2.5 g of PEG and 10 ml distilled water cooled at 4°C) and PBS (2.5 g and 10 ml of phosphate buffer pH7.4 at room temperature). The prepared solutions were stored at a temperature of 4˚C, with two-week stability. After that, they were prepared freshly.

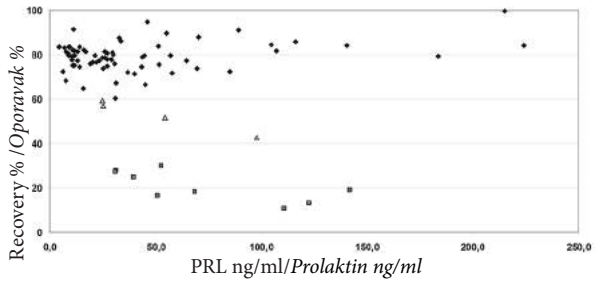
Serum samples were mixed with the equal volume of PEG solution (250 µl of serum and 250 µl of PEG solution), vortexed for one minute and then centrifuged for 30 minutes at 1800 rpm. After the precipitation of the sample, the obtained supernatant was used for laboratory determination of PRL level.

Thereafter, the recovery values were calculated according to the following formula for all precipitation modes:

Recovery (free PRL) % = ((free PRL*2)/Total PRL)*100

- Total PRL represents the measured value for serum PRL, before precipitation;
- Free PRL represents the measured value for PRL in supernatant after precipitation.

The calculated recovery value lower than 40%, is considered to be a positive finding and confirms macroprolactinemia, whereas the recovery value of 61% and higher clearly excludes macroprolactinemia. However, values ranging from 41 to 60% are considered to be a "gray area" and can be interpreted as poorly positive results for macroprolactinemia [7].



Graph 1. Distribution of macroprolactinemia (Re%) in relation to the initial blood prolactin concentrations
Grafikon 1. *Distribucija makroprolaktinemije (Re%) u odnosu na početne vrednosti prolaktina*
Legend: x-axis: initial values of prolactin in ng/ml; y-axis: recovery values in %
Legenda: x-osa: početne vrednosti prolaktina (ng/ml); y-osa: Rikaveri vrednosti (%)

Table 1 shows PRL concentrations obtained from fresh and pre-frozen aliquots, treated with PEG solution. The PRL levels were lowest in the fresh serum supernatants which were treated with phosphate solution of PEG, and highest in the supernatants of previously frozen serum treated with aqueous solution of PEG.

The lowest recovery values were obtained from the serum supernatant treated with PEG dissolved in PBS, while the highest values were obtained from the supernatant of the previously frozen serum samples treated with an aqueous solution of PEG **(Table 2)**. The statistical analysis did not show a significant difference between the percentage recovery values (Re%) obtained by precipitation of fresh and frozen serum samples using PEG dissolved in PBS ($p = 0.893$). However, there was a statistically significant difference between the calculated Re% values using aqueous solution of PEG before and after freezing the serum samples ($p = 0.03$). Also, no difference was noticed when comparing the calculated Re% value obtained

Table 1. Prolactin levels measured in the supernatants versus all four precipitation modalities
Tabela 1. *Vrednosti prolaktina izmerene u supernatantu u odnosu na sva četiri modaliteta precipitacije*

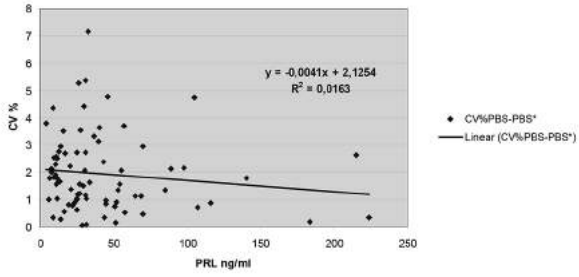
N/Broj = 82	PEG-PBS	PEG-H_2O	PEG-PBS*	PEG-H_2O*
Mean/*Srednja*	15.63	16.66	15.81	16.91
SD/*Standardna devijacija*	18.98	20.56	19.52	20.54
Median/*Medijana*	9.59	10.06	9.85	10.46
Min/*Minimum*	1.75	1.78	1.59	1.65
Max/*Maksimum*	109.44	116.33	115.12	115.34
95% CI/*Interval pouzdanosti*	7.35 – 11.40	7.97 – 12.10	7.57 – 11.26	8.32 – 11.98

Legend: PEG-PBS - supernatant prolactin levels of fresh serum treated with PEG dissolved in PBS; PEG-H_2O - supernatant of fresh water-soluble PEG; PEG-PBS* - supernatant of frozen serum treated with PEG dissolved in PBS; PEG-H_2O* - supernatant of the frozen serum treated with water-soluble PEG
Legenda: PEG-PBS – vrednost prolaktina izmerena u supernatantu svežeg seruma tretiranog polietilenglikolom rastvorenim u fosfatnom puferu; PEG-H_2O - vrednost prolaktina izmerena u supernatantu svežeg seruma tretiranog polietilenglikolom rastvorenim u vodi; PEG-PBS - vrednost prolaktina izmerena u supernatantu prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u fosfatnom puferu; PEG-H_2O* - vrednost prolaktina izmerena u supernatantu prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u vodi*

Normal distribution of continuous variables was assessed with the Shapiro-Wilk test. Data were presented as mean ± standard deviation (SD) for normally distributed continuous variables and median for non-parametric continuous variables. Parametric (t-test) and non-parametric (Mann-Whitney) statistical tests were used. A complete statistical analysis was done using Data Analysis Excel (Microsoft Corp, Redmond, WA). The results are shown in tables and figures. Differences were considered significant if $p < 0.05$ (2-tailed).

Results

This study included 82 patients who had low, normal or high PRL levels (compared to the manufacturer's reference levels). Of the total number of patients, macroprolactinemia was established in 10 patients. The distribution of macroprolactinemia in relation to the initial basal PRL levels is shown in **Graph 1**. In regard to PRL levels, the patients with laboratory-established macroprolactinemia had slightly elevated (~ 30 ng/ml) to high levels, but they did not exceed 150 ng/ml.



Graph 2. Linear regression between the concentrations of duplicate measurements of prolactin in the supernatant, before and after the freezing cycle
Grafikon 2. *Linearna regresija između koncentracija duplikatnog merenja prolaktina dobijenog iz supernatanta, svežih i prethodno zamrzavanih uzoraka seruma*
Legend: x-axis: PRL - prolactin values in ng/ml; y-axis: CV% - Coefficient of variation in %
Legenda: x-osa: PRL – vrednosti prolaktina (ng/ml); y-osa: CV% – koeficijent varijacije u %; PBS - fosfatni pufer

Table 2. Recovery values (Re%) obtained from the supernatant by precipitation with aqueous and phosphate solution of PEG, before and after freezing the serum samples
Tabela 2. *Rikaveri vrednosti (Re%) dobijene primenom polietilenglikola (PEG) rastvorenog u vodi i fosfatnom puferu (PBS), pre i posle zamrzavanja seruma*

N/*Broj* = 82	Re PEG-PBS	Re PEG-H_2O	Re PEG-PBS*	Re PEG-H_2O*
Mean/*Srednja*	71.56	75.16***	71.49*	76.49**
SD/*Standardna devijacija*	19.97	21.33	19.61	21.06
Median/*Mediana*	77.77	82.05	77.33	81.81
Min/*Minimum*	10.59	11.05	11.75	12.91
Max/*Maksimum*	101.80	115.65	107.08	108.06
95% Confidence interval/*Interval pouzdanosti*	75.85 - 79.72	78.24 - 84.13	75.01 - 78.61	79.78 - 84.03

Legend: Re PEG-PBS - recovery value calculated from fresh serum treated with PEG dissolved in PBS; Re PEG-H_2O - recovery value calculated from fresh water-treated PEG supernatant; Re PEG-PBS* - recovery value calculated from the supernatant of frozen serum treated with PEG dissolved in PBS; Re PEG-H_2O* - recovery value calculated from the supernatant of frozen serum treated with water-soluble PEG;
* Difference between Re% values calculated from the supernatant of fresh vs. frozen serum samples treated with PEG dissolved in PBS, p = 0893; ** Difference between Re% values calculated from the supernatant of fresh vs. frozen serum samples treated with PEG dissolved in water, p = 0.03; *** Difference between Re% values calculated from the supernatant of fresh serum samples treated with PEG dissolved in PBS compared to one dissolved in water, p = 0.000
Legenda: Re PEG-PBS – Rikaveri vrednost dobijena na osnovu vrednosti izmerenog prolaktina u supernatantu svežeg seruma tretiranog polietilenglikolom rastvorenim u fosfatnom puferu; Re PEG-H_2O – Rikaveri vrednost dobijena na osnovu vrednosti izmerenog prolaktina u supernatantu svežeg seruma tretiranog polietilenglikolom rastvorenim u vodi; PEG-PBS – Rikaveri vrednost dobijena na osnovu vrednosti izmerenog prolaktina u supernatantu prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u fosfatnom puferu; PEG-H_2O* – Rikaveri vrednost dobijena na osnovu vrednosti izmerenog prolaktina u supernatantu prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u vodi*
** Razlika između rikaveri vrednosti izračunatih iz supernatanta uzoraka svežeg i prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u fosfatnom puferu, p = 0893; ** Razlika između rikaveri vrednosti izračunate iz supernatanta uzoraka svežeg i prethodno zamrzavanog seruma tretiranog polietilenglikolom rastvorenim u vodi, p = 0,03; *** Razlika između rikaveri vrednosti izračunatih iz supernatanta svežih uzoraka seruma tretiranih polietilenglikolom rastvorenim u fosfatnom puferu i vodi, p = 0,000*

by treating the serum with buffer solution of PEG with respect to the values obtained by applying an aqueous solution of PEG (p = 0.000) **(Table 2).**

For the purpose of testing the reproducibility of the precipitation method with PEG prepared with phosphate buffer, the percentage of the coefficient of variation (CV%) for each of the subjects was calculated. The deviation of duplicate measurements in the supernatant, before and after freezing, was normalized in relation to the initial value of PRL. The mean value of the CV% is 1.95 ± 1.40%.

The analysis of linear regression monitored the ratio of CV% in each subject, relative to the levels of PRL prior to precipitation by PEG dissolved in PBS. The results showed that there was no statistically significant difference between the calculated values of the CV% and the initial concentrations of PRL **(Graph 2).**

Discussion

In a total of 82 subjects (with low, normal, elevated and high values of PRL) macroprolactinemia was found in 10 patients (12.2%). According to the findings of this research, the obtained percentage of laboratory confirmed macroprolactinemia is in agreement with other published data [2]. If a laboratory test is performed in the population of healthy people, using some of the available commercial tests, large molecules of PRL account for about 10% [8]. Immunoassays for determination of PRL concentration, which are used in everyday laboratory practice, have satisfactory analytical sensitivity, but the final results are significantly different depending on the applied method. The leading reason for this is the heterogeneity of the PRL molecule itself, which often results in a misinterpretation of the laboratory tests [9]. For this reason, in a number of cases, the percentage of large macromolecules varies significantly and can reach up to 90% of total PRL serum concentration [8].

The macroprolactinemia was calculated as percentage of PRL recovery. A recovery value less than 40% was taken as a cut off value to confirm significant macroprolactinemia [7]. However, in the interpretation of laboratory results using PEG, there are recommendations that rely solely on the fall of the PRL values and of large macromolecules after precipitation [10]. The obtained PRL values after precipitation have to be compared with the previously established refractory values of PRL after precipitation with PEG in the healthy population. Interpretation of the results in this way prevents the possibility of confirmation of macroprolactinemia in situations where the values of PRL after PEG precipitation are still elevated or very high. However, patients included in this study did not have very high initial values of PRL. The values after precipitation were significantly lower, and corresponded to gender-specific reference values.

As this method is insufficiently specific, in addition to PRL macromolecules, monomeric, bio-

logically active forms of this hormone are also being precipitated [10, 11]. According to the results of this study, in patients with laboratory confirmed macroprolactinemia, serum PRL values prior to precipitation ranged from 31.0 to 142.0 pg/ml **(Graph 1)**. The concentrations of PRL prior to precipitation by PEG solution were in the range of slightly or moderately elevated, according to the reference interval recommended by the manufacturer. The measured values of PRL, in patients in whom macroprolactinemia was determined, correspond to the range of elevated PRL levels which, according to the recommendation of the Guide for pituitary gland disease, must be further analyzed for the presence of macroprolactinemia [12]. On the other hand, the range of PRL serum levels in which macroprolactinemia has not been established is broad, ranging from low to very high levels of PRL, which corresponds to most of the published results so far [1, 9].

Macroprolactinemia is considered a benign condition, with rare clinical symptoms and signs of hyperprolactinemia and as such it does not require additional laboratory and/or morphological diagnostic procedures, medical or surgical treatment or further long-term monitoring.

Precipitation with PEG solution is a reliable, simple screening test for macroprolactinemia. Insufficient literature data on the precipitating method itself has led us to perform an examination in relation to the type of PEG solution. In the vast majority of available papers, this information is ignored, which gives the impression that the reliability of the method is the same regardless of the type of solvent used. According to the literature data, an aqueous PEG solution is used as a PRL precipitating agent in approximately 59% of cases in everyday laboratory practice [13].

According to the manufacturer's recommendations, PEG can be dissolved in water and PBS. One of the objectives of the study was to determine whether the precipitation with PEG dissolved in phosphate buffer or in water has a higher analytical value in the detection of macroprolactinemia. Based on the obtained results, the PEG solution prepared with PBS was a more efficient and safer modality in the laboratory detection of macroprolactinemia, compared to the aqueous PEG solution **(Table 1)**.

The specific nature of laboratory work imposes an appropriate schedule and frequency of performing laboratory analysis, as well as the preservation of the serum samples themselves. Since the method itself requires personal engagement concerning the manual pretreatment of the sample, the time required for centrifugation and obtaining the final supernatant, in order to determine the level of PRL, dictates the frequency of analysis. This was the main reason for comparing the macroprolactinemia values in relation to the moment of performing precipitation by treating fresh, unfrozen, opposite to previously frozen serum samples. According to the obtained results, the PEG solution prepared with PBS represents an equally reliable modality in the laboratory determination of macroprolactinemia regardless of whether fresh or previously frozen serums are used. However, this relation has not been established for the application of aqueous solution of PEG. Therefore, if this precipitating method of macroprolactinemia detection is not performed on a daily basis, phosphate buffer is recommended as a solvent for the preparation of PEG.

For the purpose of reproducibility testing of the precipitation method using PEG dissolved in PBS, percentage coefficients of variation (CV%) were calculated for each subject. The CV% represents the deviation of duplicate PRL measurements in the supernatants obtained from fresh and previously frozen serum samples (CV% is normalized to the initial value of PRL). The mean CV% value (1.95 ± 1.40%) is preferably low, indicating a high degree of reproducibility in macroprolactinemia determination of all PRL levels. The study has shown that the level of precipitation does not depend on initial PRL concentrations, therefore PRL concentration does not affect the calculated recovery value in areas of low, elevated or high PRL values **(Graph 2)**. Accordingly, precipitation method with PEG provides a reliable laboratory confirmation of macroprolactinemia, regardless of the level of serum PRL measured before the precipitation.

Previous findings show that the results of the laboratory detection of macroprolactinemia, obtained by the precipitation method using PEG, are highly correlated with the results obtained by gel filtration chromatography [14, 15]. However, it is considered that the above precipitation method also underestimates the values of the monomeric PRL, which is responsible for the biological effects by about 25% [8, 16, 17].

Limitations of the study

In the light of the obtained results, it should be noted that the applied precipitation method was not compared to other laboratory methods, such as gel filtration chromatography, which represents a gold standard in the detection of macroprolactinemia. Furthermore, another limitation of the study is the relatively small sample size.

Conclusion

According to the results, laboratory determination of macroprolactinemia based on polyethylene glycol precipitation is a reproducible method**.**

The usage of polyethylene glycol dissolved in phosphate buffered saline allows determination of macroprolactinemia with equal reliability in low, elevated and high prolactin levels using fresh, unfrozen as well as previously frozen and stored serum samples at - 20°C.

In order to rationalize daily laboratory performance, determination of macroprolactinemia using polyethylene glycol dissolved in phosphate buffered saline may be performed in smaller series.

References

1. Shimatsu A, Hattori N. Macroprolactinemia: diagnostic, clinical and pathogenic significance. Cin Dev Immunol. 2012; 2012:167132.

2. Hauache OM, Rocha AJ, Maia ACM, Maciel RMB, Vieira JGH. Screening for macroprolactinemia and pituitary imaging studies. Clin Endocrinol (Oxf). 2002;57(3):327-31.

3. Kasum M, Oreskovic S, Zec I, Jezek D, Tomic V, Gall V, at al. Macroprolactinemia: new insights in hyperprolactinemia. Biochem Med (Zagreb). 2012;22(2):171-9.

4. Kasum M, Pavičić-Baldani D, Stanić P, Orešković S, Sarić JM, Blajić J, et al. Importance of macroprolactinemia in hyperprolactinemia. Eur J Obstet Gynecol Reprod Biol. 2014;183:28-32.

5. Donadio F, Barbieri A, Angioni R, Mantovani G, Beck-Peccoz P, Spada A, et al. Patients with macroprolactinaemia: clinical and radiological features. Eur J Clin Invest. 2007;37(7):552-7.

6. Lippi G, Plebani M. Macroprolactin: searching for a needle in a haystack? Clin Chem Lab Med. 2016;54(4):519-22.

7. Gibney J, Smith TP, McKenna TJ. The impact on clinical practice of routine screening for macroprolactin. J Clin Endocrinol Metab. 2005; 90(7):3927-32.

8. Suliman AM, Smith TP, Gibney J, McKenna TJ. Frequent misdiagnosis and mismanagement of hyperprolactinemic patients before the introduction of macroprolactin screening: application of a new strict laboratory definition of macroprolactinema. Clin Chem. 2003;49(9):1504-9.

9. Hattori N, Adachi T, Ishihara T, Shimatsu A. The natural history of macroprolactinemia. Eur J Endocrinol. 2012;166(4):625-9.

10. Fahie-Wilson M, Halsall D. Polyethylene glycol precipitation: proceed with care. Ann Clin Biochem. 2008;45(Pt 3):233-5.

11. Fahie-Wilson M, Smith TP. Determination of prolactin: the macroprolactin problem. Best Pract Clin Endocrinol Metab. 2013;27(5):725-42.

12. McKenna TJ. Should macroprolactin be measured in all hyperprolactinaemic sera? Clin Endocrinol. 2009;71(4):466-9.

13. Boscato L, Scott S. Endocrine working party macroprolactin - what do you do [abstract]. Clin Biochem Rev. 2012;33:S28.

14. Kavanagh L, McKenna TJ, Fahie-Wilson MN, Gibney J, Smith P. Specificity and clinical utility of methods for detection of macroprolactin. Clin Chem. 2006;57(7):1366-72.

15. Whitehead SJ, Cornes MP, Ford C, Gama R. Reference ranges for serum total and monomeric prolactin for the current generation Abbott Architect assay. Ann Clin Biochem. 2015;52 (Pt 1):61-6.

16. McCudden CR, Sharpless JL, Grenache DG. Comparison of multiple for identification of hyperprolactinemia in the presence of macroprolactin. Clin Chim Acta. 2010;411(3-4):155-60.

17. Chen Y, Wang H, Yang W, Jin W, Yu W, Wang W, et al. A new method of using polyethylene glycol (PEG) precipitation of macroprolactin to detect genuine hyperprolactinemia. J Clin Lab Anal. 2016;30(6):1169-74.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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University of Novi Sad, Faculty of Medicine Novi Sad[2]

Case report
Prikaz slučaja
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WARM ANTIBODY AUTOIMMUNE HEMOLYTIC ANEMIA IN PREGNANCY − A CASE REPORT

AUTOIMUNA HEMOLIZNA ANEMIJA SA TOPLIM ANTITELIMA U TRUDNOĆI − PRIKAZ SLUČAJA

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Summary

Introduction. Warm autoimmune hemolytic anemia is the presence of warm autoantibodies against red blood cell with or without complement activation. The presence of warm autoantibodies on the red blood cells is detected by direct antiglobulin test with polyspecific and immunoglobulin G reagents. Antibodies removed from the red blood cells tested by indirect antiglobulin test show panagglutination with a panel of red blood cells. **Case Report.** We report a rare case of idiopathic warm autoimmune hemolytic anemia in a 26-year-old woman in the early pregnancy. Warm autoimmune hemolytic anemia was mild, so during monitoring the risk to the fetus was assessed as low. The fetal status was assessed every four weeks. The noninvasive Doppler examination of the fetal middle cerebral artery revealed no fetal anemia. The last control before childbirth was done in the 38 week of pregnancy and the fetal direct antiglobulin test was 4+ and indirect antiglobulin test was 2+. The newborn presented with warm autoantibody immunoglobulin G, and positive direct antiglobulin test (3+). The infant was breastfed for nine months after birth. The direct antiglobulin tests were positive (3+) in both mother and child over the following 12 months. **Conclusion.** In case of warm autoimmune hemolytic anemia, the main purpose is to stop hemolysis and correct anemia in pregnant women, but it is also necessary to monitor the fetal condition in order to detect fetal hemolytic anemia as early as possible.

Key words: Anemia, Hemolytic, Autoimmune; Pregnancy Complications, Hematologic; Autoantibodies; Prenatal Exposure Delayed Effects; Breast Feeding; Pregnancy; Fetal Monitoring

Sažetak

Uvod. Topla autoimuna hemolizna anemija definisana je postojanjem toplih antitela protiv sopstvenih eritrocita, sa aktivacijom komplementa ili bez nje. Prisustvo toplih autoantitela na pacijentovim eritrocitima otkriva se direktnim antiglobulinskim testom sa polispecifičnim i imunoglobulin G reagensima. Antitela uklonjena sa pacijentovih eritrocita pokazuju u indirektnom antiglobulinskom testu panaglutinaciju sa panelom test eritrocita. **Prikaz slučaja.** Izveštavamo o retkom slučaju idiopatske tople autoimune hemolizne anemije koja se pojavila početkom trudnoće kod 26-godišnje žene. Topla autoimuna hemolizna anemija je bila blaga, pa je rizik po fetus u toku praćenja stanja fetusa ocenjen kao nizak. Status fetusa kontrolisan je svake četiri nedelje. Neinvazivnom doplerometrijom srednje cerebralne arterije fetusa nije otkrivena fetalna anemija. Na poslednjoj kontroli trudnice pre porođaja, koja je izvršena u 38. nedelji trudnoće, bio je direktni antiglobulinski test 4+a, indirektni antiglobulinski test 2+. Kod novorođenčeta je otkriveno toplo autoantitelo klase imunoglobulina G i pozitivan direktni antiglobulinski test (3+). Dojenče je dojilo devet meseci nakon rođenja. I kod majke i kod deteta direktni antiglobulinski test ostao je pozitivan (3+) u narednih 12 meseci. **Zaključak.** Glavni cilj je zaustavljanje hemolize i otklanjanje anemije kod buduće majke. Kod trudnica sa toplom autoimunom hemoliznom anemijom neophodno je i praćenje stanja fetusa kako bi se što pre otkrila hemolizna anemija fetusa.

Ključne reči: autoimuna hemolitička anemija; hematološke komplikacije u trudnoći; autoantitela; prenatalna izloženost odloženi efekti; dojenje; trudnoća; fetalni monitoring

Introduction

Autoimmune hemolytic anemia (AIHA) is defined as the increased destruction of circulating red blood cells

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Abbreviations
AIHA – autoimmune hemolytic anemia
RBCs – red blood cells
IgG – immunoglobulin G
DAT – direct antiglobulin test
WAIHA – warm autoimmune hemolytic anemia
IAT – indirect antiglobulin test
LISS – low ionic strength solution

(RBCs) in the presence of anti-RBC autoantibodies with or without complement activation [1]. Warm autoantibodies are typically immunoglobulin G (IgG) antibodies whose optimal reaction temperature is 37°C [2].

The presence of warm autoantibody on the patient's cells is shown by a positive direct antiglobulin test (DAT) with polyspecific and IgG reagents. The presence of a free warm autoantibody in the patient's serum is shown by reactivity with all panel cells. Stronger reactivity against some panel cells implies the presence of underlying antibody [3, 4]. The presence of hemolytic anemia is indicated by laboratory evidence of RBCs destruction (low hemoglobin, elevated bilirubin level and lactate dehydrogenase (LDH), RBCs shape changes on the peripheral blood smear etc.) [5]. The main purpose of AIHA treatment is to stop hemolysis and correct anemia.

Warm autoantibodies are detected in 1: 1,000 to 1:50,000 of pregnancies. The pregnant women should be evaluated for RBCs hemolysis. If there is no hemolysis, they may be managed similar to pregnant women without RBCs antibodies. Autoimmune hemolysis in pregnancy from a combination of warm and cold

Table 1. An overview of laboratory test results
Tabela 1. *Pregled rezultata laboratorijskih ispitivanja*

Laboratory tests *Laboratorijski testovi*	Patient's results *Rezultati pacijenta*		Reference interval *Referentni interval*
WBC (White Blood Cell) x10^9/L/*Broj belih krvnih ćelija x10^9/L*	8.8	Normal/*Normalan*	4–10
RBC x 10^{12}/L (Red Blood Cell)/*Broj crvenih krvnih ćelija x10^{12}/L*	3.1	Low/*Nizak*	3.8–6
Hemoglobin g/L/*Hemoglobin g/L*	105	Low/*Nizak*	120–180
HCT - Hematocrit %/*Hematokrit %*	0.31	Low/*Nizak*	0.35–0.54
MCV (Mean Corpuscular Volume) fL *Srednji volumen crvenih krvnih ćelija fL*	99.4	High/*Visok*	80–97
MCH (Mean Corpuscular Hemoglobin) fmol *Srednji korpuskularni hemoglobin fmol*	30.4	Normal/*Normalan*	26.5–33.5
MCHC (Mean Corpuscular Hemoglobin Concentration) g/L *Srednja korpuskularna koncentracija hemoglobina g/L*	348	Normal/*Normalan*	315–360
RDW (Red Cell distribution Width) % *Širina distribucije crvenih krvnih ćelija %*	16.6	High/*Visok*	10–15
Reticulocyte count %/*Broj retikulocita %*	4.5	High/*Visok*	0.5–2.5
Haptoglobin/*Haptoglobin*	0.1	Low/*Nizak*	0.3 – 2.0
Platelet count x10^9/L/*Broj trombocita x10^9/L*	184	Normal/*Normalan*	120–450
MPV (Mean Platelet Volume) fL/*Srednja zapremina trombocita fL*	9.9	Normal/*Normalan*	6.5–11
PCT (Plateletcrit) %/*Trombokrit %*	0.18	Normal/*Normalan*	0.5
PDW (Platelet Distribution Width) %/*Širina distribucije trombocita %*	15.8	Normal/*Normalan*	10–18
Lymphocytes %/*Limfociti %*	37.7	Normal/*Normalan*	20–40
Monocytes %/*Monociti %*	8.5	Normal/*Normalan*	0.5–10
Neutrophil granulocytes %/*Neutrofilni granulociti %*	52.3	Normal/*Normalan*	50–70
Eosinophilic granulocytes %/*Eozinofilni granulociti %*	1.3	Normal/*Normalan*	0–4
Basophilic granulocytes %/*Bazofilni granulociti %*	0.2	Normal/*Normalan*	0–2
Peripheral blood smear *Razmaz periferne krvi %*	Macrocytosis and polychromasia *Makrocitoza i polihromazija*		
Bilirubin, total µmol/L/*Ukupni bilirubin µmol/L*	5.1	Normal/*Normalan*	5–21
Biliruibin, conjugated µmol/L/*Konjugovani bilirubin µmol/L*	3.6	High/*Visok*	0–3.4
AST - Aspartate transaminase U/L/*Aspartat transaminaza U/L*	19	Normal/*Normalan*	0–35
ALT - Alanine transaminase U/L/*Alanin transaminaza U/L*	13	Normal/*Normalan*	0–45
GGT- Gamma glutamyltransferase U/L/*Gama glutamiltransferaza U/L*	13	Normal/*Normalan*	0–55
LDH - *Lactate dehydrogenase* U/L/*Laktat dehidrogenaza U/L*	420	High/*Visok*	208–378
Fibrinogen g/L/*Fibrinogen g/L*	4.38	High/*Visok*	1.7–4
Serum iron mmol/L/*Serumsko gvožđe mmol/L*	15	Normal/*Normalan*	14,4–25,1
Ferritin *ng/mL/Feritin ng/mL*	35	Normal/*Normalan*	30–150

autoantibodies had been estimated to occur in 1 in 50,000 pregnancies [6]. Since IgG can cross the placenta, the antibody may be detectable in the infant's serum and/or attached to infant's cells. Hemolytic disease of the fetus and newborn has been reported [7].

Warm autoimmune hemolytic anemia (WAIHA) caused primarily by pregnancy is rarely reported in the literature [6]. We present a case of idiopathic WAIHA in the early pregnancy of a 26-year-old woman. The WAIHA was mild and did not require active treatment. The risk to the infant was assessed as low during monitoring the fetal condition in order to identify fetal hemolytic anemia as early as possible. The aim of this report was to describe the clinical presentation, diagnostic investigations, and possible outcomes of pregnancy in WAIHA.

Case Report

A 26-year-old woman, who was pregnant for the first time, visited the Blood Transfusion Institute of Vojvodina for routine RBC antibody screening. The woman was twelve weeks pregnant and reported no significant past medical history. She had never had a transfusion before, or abortion of an unintended pregnancy. In her recent medical history, she was not on any medication.

On initial testing, it was determined that the woman had a blood type B, Rh-D-negative, Rh phenotype ccddee, Kell phenotype Kk. The routine antenatal maternal antibody screening was performed using the indirect antiglobulin test (IAT), gel technique on commercial low ionic strength solution (LISS)/Coombs cards containing anti-IgG and anti-C3d (ID-Card LISS/Coombs, BioRad, Cressier, Switzerland) with commercial test RBC (ID-DiaCell I-II, BioRad, Cressier, Switzerland). Testing was performed using an automated immunoassay analyzer (IH-500, BioRad). The IAT was positive (2+). The antibody specificity was determined by IAT using commercially available 11-cell panel typed for all clinically relevant antigens (ID-DiaPanel, BioRad, Cressier, Switzerland) in LISS/Coombs gel cards. The antibody in the patient's serum has shown reactivity of the same intensity with all panel cells. The autocontrol was positive (2+).

The DAT in LISS/Coombs gel card was positive (2+). The aim of performing DAT in LISS/Coombs gel card (DC-Screening I, BioRad, Cressier, Switzerland) was to determine whether IgG, IgA, IgM, C3c, and C3d were bound to RBCs, and DAT was positive only for IgG.

After removal of the antibody from the RBCs using heat elution technique, the eluate was subsequently tested against a commercially available 11-cell panel by IAT. Panagglutination was detected, confirming the presence of a warm autoantibody.

It was determined that the potential father had a blood type O, Rh-D positive, Rh phenotype CcDee, Kell phenotype Kk.

The subsequent specimen, taken 4 weeks later, was tested in the same way. Antibody identification cell panel showed strong panagglutination warm autoantibody. Four weeks later, at 20 weeks gestation, the laboratory result was the same, but the pregnant patient presented with fatigue and dizziness so she was referred to a hematologist for further evaluation. She was mildly anemic **(Table 1)** so prenatal vitamins and iron were included in her therapy, without requiring hospitalization. The last control before childbirth, which was done at 38 weeks of pregnancy, showed DAT 4+, IAT 2+. The hemoglobin level remained unchanged throughout the pregnancy.

The fetal status was evaluated every four weeks. Fetal anemia was not detected using a non-invasive fetal middle cerebral arterial Doppler.

The woman delivered a term male infant at 40 weeks gestation by Caesarean section. The infant presented with Apgar scores of 10 and 10, had no jaundice or anemia and had type O Rh-D-negative with a positive DAT (3+). Elution studies were positive for the maternal warm autoantibody only. Hemoglobin level was 16.2 g/dL (pediatric reference ranges 13.4 – 19.9 g/dL for 0 – 1 month) [8]. The infant was discharged after 3 days in stable condition.

After we assessed that the benefit of breastfeeding would be greater than the risk for the infant, the infant was breastfed for nine months after birth. The DATs were positive (3+) in both mother and child over the following 12 months, but neither needed RBC transfusion. The child's hemoglobin remained stable for 12 months of follow up. The mother's anemia went into spontaneous remission several months later.

Discussion

The AIHA may be primary or secondary. No underlying disease or agent can be detected in primary AIHA. The secondary causes of AIHA include lymphomas, Chronic Lymphocytic Leukemia, solid tumors, Systemic Lupus Erythematosus, antiphospholipid syndrome, Sjogren's syndrome, rheumatoid arthritis, drug abuse [9].

In the present case, the autoantibody was not associated with other autoimmune diseases or malignancies. The warm autoantibody was pregnancy associated. The pregnant woman had a mild autoimmune hemolytic anemia and the basic question during pregnancy monitoring was whether the passage of maternal autoantibodies will pose a risk to the child. Due to the fact that fetal anemia was not detected, we decided only to maintain careful monitoring. As the healthy term infant was without anemia and jaundice, the next question was the risk and potential benefits of breastfeeding for the child. Currently, breastfeeding is accepted as a very effective primary health care strategy for improving infant health, as well as lowering the risk of a significant number of chronic diseases in older children and adults [10]. For these reasons, we recommended breastfeeding and monitoring the baby's condition during this period.

Pregnancy-induced WAIHA is rarely described in the literature, but in 1982, a group of authors confirmed an association between erythrocyte autosensitization and pregnancy in the largest series of 20 pregnant women with RBC autoantibodies. The clinical manifestation varied from severe to mild hemolytic anemia.

Three infants were mildly affected with hemolytic disease due to the maternal autoantibodies crossing the placenta but no treatment was needed [11].

In case of WAIHA, it is important to detect the presence of an underlying alloantibody when a transfusion is contemplated. The RBC transfusions and corticosteroids (e.g. Prednisone 1 – 1.5 mg/kg/day) can be administered to stabilize hematocrit and to control hemolysis as well as intravenous immunoglobulin or plasma exchange. Other therapy (splenectomy, immunosuppressive, Danazol) is used in the treatment of non-pregnant patients [12].

Conclusion

In warm autoimmune hemolytic anemia, the main purpose is to stop hemolysis and correct anemia in pregnant women. Fetal monitoring in pregnant women with warm autoimmune hemolytic anemia is necessary in order to identify fetal hemolytic anemia as early as possible.

References

1. Barcellini W. New insights in the pathogenesis of autoimmune hemolytic anemia. Transfus Med Hemother. 2015;42(5):287-93.

2. Sürücü G, Mayer B, Märzacker A, Yürek S, Salama A. Harmless pregnancy-induced warm autoantibodies to red blood cells. Transfus Med Hemother. 2015;42(5):325-7.

3. Hill A, Hill QA. Autoimmune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2018;2018(1):382-9.

4. Jäger U, Barcellini W, Broome CM, Gertz MA, Hill A, Hill QA, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. Blood Rev. 2020;41:100648.

5. Park SH. Diagnosis and treatment of autoimmune hemolytic anemia: classic approach and recent advances. Blood Res. 2016;51(2):69-71.

6. Gavva C. Warm autoantibodies during pregnancy. In: Nester T, editor. Transfusion management of the obstetrical patient. Cham: Springer; 2018. p. 201-6.

7. Ladogana S, Maruzzi M, Samperi P, Perrotta S, Del Vecchio GC, Notarangelo LD, et al. Diagnosis and management of newly diagnosed childhood autoimmune haemolytic anaemia. Recommendations from the Red Cell Study Group of the Paediatric Haemato-Oncology Italian Association. Blood Transfus. 2017;15(3):259-67.

8. Fulgoni VL 3rd, Agarwal S, Kellogg MD, Lieberman HR. Establishing pediatric and adult RBC reference intervals with NHANES data using piecewise regression. Am J Clin Pathol. 2019;151(2):128-42.

9. Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A, and al. The diagnosis and management of primary autoimmune haemolytic anaemia. Br J Haematol. 2017;176(3):395-411.

10. American Academy of Pediatrics. Breastfeeding and the use of human milk. Section on breastfeeding. Pediatrics. 2012;129(3):e827-41.

11. Sokol RJ, Hewitt S, Stamps BK. Erythrocyte autoantibodies, autoimmune haemolysis and pregnancy. Vox Sang. 1982;43(4):169-76.

12. Dara RC, Tiwari AK, Arora D, Mitra S, Acharya DP, Aggarwal G, et al. Alloimmunization in autoimmune hemolytic anemia patient: The differential adsorption approach. Asian J Transfus Sci. 2017;11(1):53-7.

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DE GARENGEOT'S HERNIA – A CASE OF INCARCERATED FEMORAL HERNIA WITH ACUTE GANGRENOUS APPENDICITIS AND LITERATURE REVIEW

DE GARENGEOVA HERNIJA – PRIKAZ SLUČAJA INKARCERIRANE FEMORALNE KILE SA AKUTNIM GANGRENOZNIM APENDICITISOM I PREGLEDOM LITERATURE

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Summary
Introduction. De Garengeot's hernia is a rare type of femoral hernia that contains the appendix within the hernia sac and it is found in 0.5–5% of cases. The incidence of appendicitis within the de Garengeot's hernia is 0.08–0.13%. We present a case of de Garengeot's hernia with a gangrenous appendicitis and an extensive literature review of published cases. **Case Report.** We present a case of a 68-year-old woman who underwent urgent surgery due to an incarcerated femoral hernia and preoperatively undiagnosed de Garengeot's hernia with a gangrenous appendicitis within the femoral hernia sac. **Conclusion.** De Garengeot's hernia is most commonly diagnosed intraoperatively and requires immediate surgery in order to avoid complications. There are no recommendations regarding the choice of surgical procedure for femoral defect repair.
Key words: Hernia, Femoral; Appendicitis; Gangrene; Appendectomy; Laparoscopy; Surgical Procedures, Operative; Surgical Mesh; Diagnosis; Diagnostic Imaging; Groin

Sažetak
Uvod. De Garengeova kila je redak oblik femoralne kile sa apendiksom unutar kilne kese i može se naći kod 0,5–5% slučajeva. Incidencija apendicitisa u De Garengeovoj herniji je 0,08–0,13%. Prikazujemo slučaj De Garengeove hernije sa gangrenoznim apendicitisom i pregled literature publikovanih slučajeva. **Prikaz slučaja.** Prikazujemo slučaj 68-godišnje žene koja je hitno operisana zbog inkarcerirane femoralne kile sa preoperativno nedijagnostikovanom De Garengeovom kilom i gangrenoznim apendiksom u femoralnoj kilnoj kesi. **Zaključak.** De Garengeova kila se najčešće dijagnostikuje intraoperativno i zahteva hitnu operaciju kako bi se izbegle komplikacije. Nema preporuka u vezi sa izborom hirurške metode za reparaciju femoralnog defekta.
Ključne reči: femoralna hernija; apendicitis; gangrena; apendektomija; laparoskopija; hirurške operativne procedure; hirurška mrežica; dijagnoza; dijagnostički imidžing; prepone

Introduction

De Garengeot's hernia is a rare type of femoral hernia with an appendix within the hernia sac and it is found in 0.5–5% of cases [1–3]. The reasons for this are low-positioned cecum, its increased mobility, and pelvic localization of the appendix. It was first described in 1731 by the French surgeon Rene Jacques Croissant de Garengeot [4]. The incidence of appendicitis in de Garengeot's hernias is 0.08–0.13% and Hevin was the first to perform an appendectomy in such a case in 1785 [5]. There are no recommendations for the most optimal surgical management of the femoral canal defect in de Garengeot's hernia with appendicitis [6, 7]. By searching the literature of the PubMed from 1980 – 2019, we found that a total of 133 Garengeot's hernia cases have been reported in 107 articles.

We present a case of preoperatively undiagnosed de Garengeot's hernia with gangrenous appendicitis within the hernia sac in a 68-year-old woman and review of management of a de Garengeot's hernia. To our knowledge, this is the first case of de Garengeot's hernia with gangrenous appendicitis in Montenegro. By researching and analyzing published case studies of de Garengeot's hernia from 1980 to 2019, we wish to contribute to the development of consensus on diagnostic and surgical procedures in order to reduce complications and make its treatment safe and more effective.

Case Report

A 68-year-old woman came to the Emergency Department with complaints of a tender bulge in the right inguinal region during the last 24 hours. She knew she had a femoral hernia, but she was delaying the proposed surgery. She reported pain in her lower right abdomen without vomiting and without changes in bowel habits. Her past medical history was unremarkable other than a previous laparotomy after cesarean section and smoking. On physical examination she was hemodynamically stable and had a ten-

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Abbreviations

US – ultrasonography
CT – computed tomography
MRI – magnetic resonance imaging
CRP – C-reactive protein
TAPP – transabdominal preperitoneal mesh repair
TEP – total extraperitoneal repair

der non-reducible swelling in the right groin region, below the inguinal ligament, measuring about 5 cm. There were no signs of peritonitis, the bowel sounds were attenuated on auscultation. The rest of the physical examination was unremarkable.

The laboratory findings were within normal range except for leukocytosis (14 x 10^9 g/L with 90% neutrophils) and C-reactive protein (CRP) (36.4 mg/L). The hemostasis was normal. The body temperature was elevated (37.9^{0}C). A strangulated femoral hernia was suspected and the patient underwent ultrasonography (US), which showed bowel contents and a small amount of fluid in the hernia sac. We decided to perform an emergency surgery.

The patient received intravenous fluid and double antibiotic therapy (metronidazole - 15 mg/kg and 1 g ceftriaxone) and was urgently taken to the operating room for incarcerated femoral hernia repair.

The femoral hernia sac was approached under spinal anesthesia using a modified McEvedy approach with a transverse incision of 2 cm above the pubic tubercle. Safe entry into the peritoneum was achieved after dissection of the rectus sheath and careful incision of the lacunar ligament to allow reduction of the hernia sac. The hernia sac was opened and the peritoneum was found inflamed. A gangrenous appendix was within the hernia sac without signs of perforation or adhesion to the hernia sac, but with a dark-colored fluid. The cecum was in the pelvis, without signs of induration.

Appendectomy was performed **(Figure 1)** and the base of appendix was closed with double polydioxanone ligature 3.0. Since the appendicitis was severe and there was fluid inside the hernia sac, hernia repair without a mesh was performed. The repair of the femoral hernia was done by placing a 1 - 0 Prolen suture between the inguinal ligament and the Cooper ligament to repair the defect. The postoperative course was uneventful. The patient was discharged on the fifth postoperative day. Histological examination of the excised appendix revealed gangrenous inflammation of the appendix. On follow-up, six months later, there were no sign of recurrence.

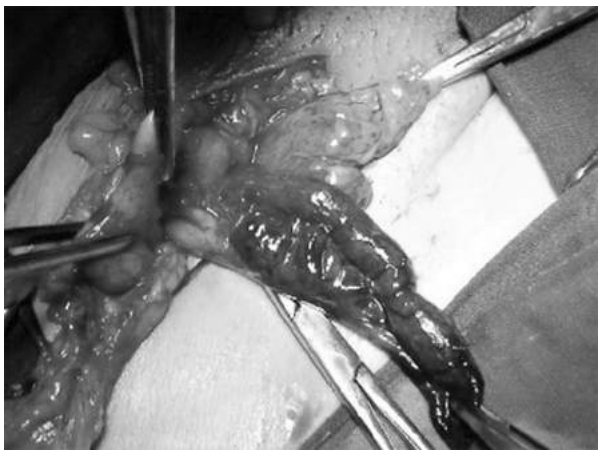

Figure 1. Intraoperative view of the inflamed appendix within the femoral hernia
Slika 1. *Intraoperativna slika inflamiranog apendiksa unutar femoralne hernije*

Discussion

Femoral hernia accounts for approximately 3% of all groin hernias and represents a protrusion of the extraperitoneal tissue, peritoneum and sometimes abdominal contents through defect in the transversalis fascia which covers the femoral ring at the entry to the femoral canal and lies within the femoral triangle [1–3]. The femoral canal is funnel-shaped, approximately 1 – 2 cm in length and 10 – 20 mm in width, bordered anterosuperiorly by inguinal ligament, medially by lacunar ligament, laterally by femoral vein, and posteriorly by pectineal ligament [4]. The femoral triangle is bordered by the inguinal ligament, sartorius muscle and adductor longus muscles and extends to the deep fascia of the thigh. The femoral ring is relatively small and rigid and represents the most proximal part of the femoral canal. It is bordered anteriorly by the inguinal ligament, posteriorly by the pectineal ligament, and laterally by the femoral vein [2–4].

Femoral hernias have an increased risk and high rate of incarceration and strangulation (15–20%) due to the small space in the femoral ring and femoral canal, and consequently the narrow neck of the femoral hernia. The femoral hernia is the third most common primary hernia, accounting for 20% of all female and 5% of male hernias, 2 times more common on the right side, and in 20% of cases it is bilateral. Risk factors for its development are smoking, chronic cough, constipation, pregnancy-related changes and older age. In 40–50% of cases, the patients only present for surgery at the stage of incarceration [4].

Femoral hernia is more common in women than men, with a 4:1 to 10:1 female-to-male ratio and usually appears with increasing age [1, 5].

Differential diagnosis of an incarcerated femoral hernia can be clinically problematic in relation to numerous benign or malignant diseases. It should be distinguished from other types of femoral hernias, lipomas, cellulitis, hypostatic abscess in retroperitoneal space, infected or metastatic lymphadenopathy, soft tissue sarcomas, femoral aneurysms**,** superficial thrombophlebitis and ectasia of the great saphenous vein, so in most cases imaging diagnostic procedures may be necessary [8]. The hernia sac contents may be omentum, preperitoneal adipose tissue, small intestine, rarely colon or bladder. In a smaller percentage of cases a portion of the circumference of the intestine (Richter), a Meckel diverticulum (Littré) or the appendix (Garengeot) may be found.

Table 1. Distribution of the histopathological findings of the appendix
Tabela 1. *Distribucija histopatološkog nalaza apendiksa*

Histopathological findings (n = 88)/*Histopatološki nalazi (n = 88)*	Number of patients n (%)/*Broj bolesnika n (%)*
Appendicitis/*Appendicitis*	51 (57.9%)
Phlegmonous appendicitis/*Flegmonozni apendicitis*	6 (6.8%)
Gangrenous appendicitis/*Gangrenozni apendicitis*	8 (9.1%)
Perforated appendicitis/*Perforativni apendicitis*	12 (13.7%)
Chronic appendicitis/*Hronični apendicitis*	3 (3.4%)
Normal appendix/*Normalan apendiks*	7 (7.9%)
Appendiceal neoplasms/*Neoplazma apendiksa*	1 (1.2%)

Garengeot's hernia is a rare type of femoral hernia with appendix in the hernia sac [9]. It was first described by the French surgeon Rene Jackues Croissant de Garengeot in 1731, who reported the first operation of a femoral hernia containing the appendix without inflammation [10].

In 2005, Akopian and Alexander first termed the femoral hernia after de Garengeot [11]. The finding of vermiform appendix in the hernia sac of the ventral abdominal hernia is extremely rare and according to literature data it accounts for approximately 1% of all cases. It is most commonly found in inguinal hernias (Amyand hernia), with an incidence of 0.19 – 1.7% and less frequently in femoral hernias (de Garengeot's hernia) with an incidence of 0.5 – 1% [4, 12–14].

We searched the PubMed database, that publishes and indexes English language medical journals for the following terms: ((de Garengeot′s hernia) OR (femoral hernia appendix) OR (French hernia) OR (crural hernia, appendix)) AND appendicitis from 2005 to 2019, and from 1980 to 2004 the following terms: ((femoral hernia, appendix) OR (French hernia) OR (crural hernia, appendix)) AND (appendicitis). The literature search showed that there are 107 articles on Garengeot's hernia, reported in 133 patients, without our case report.

There are several theories on the pathogenesis of de Garengeot's hernia. The predisposing factors for this condition are abnormal rotation of the cecum-appendix complex during embryological development with an atypical appendocecal attachment, anatomically large and low-positioned cecum, or pelvic appendix [15–19]. De Garengeot's hernia is more common in women than in men (ratio 5:1) with an age range from 29 to 91 years (mean age 70) [18]. De Garengeot's hernia commonly occurs in postmenopausal women on the right side and the predisposing factors are changes during pregnancy, smoking, constipation, increased abdominal pressure, chronic cough, muscle and connective tissue disorders, and older age [4, 20].

In the analyzed case studies, there were 22 (16.6%) men and 111 (83.4%) women. The median age of women (n = 111) was 77.3 years (range 33 – 96) and of men (n = 22) 73.7 years (range 40 – 94).

In this type of hernia, the appendix can be without alterations, but due to the incarceration that occurs in 15 – 20% of cases, congestion and varying degree of appendicitis may occur. The incidence of acute appendicitis in this type of hernia is as low as 0.08 – 0.13% [21] and perforation occurs only in approximately 0.1% of cases [12].

The first appendectomy for acute appendicitis in a patient with de Garengeot's hernia was performed by Hevin in 1787 [11]. There are several theories in recent literature about the cause of appendicitis. Most authors consider that extraluminal rather than intraluminal obstruction of the appendix is the cause of inflammation and that it is a consequence of its incarceration and strangulation in the narrow and rigid neck of the femoral ring [11, 17, 21–23]. The characteristic of acute appendicitis in de Garengeot's hernia, even in patients with a perforated appendix, is that peritonitis is rare, due to the characteristics of the neck of the femoral hernia where the appendix is isolated in the hernia sac [24].

De Garengeot's hernia should be distinguished from Amyand's hernia, where the appendix is located in the incarcerated inguinal hernia sac [16]. There is controversy in the literature regarding the use of these terms, as some authors define de Garengeot's hernia as either inguinal or femoral hernia which contains appendix without appendicitis. In the analyzed case studies, histopathological findings were obtained in 88/133 (66.2%) patients. **Table 1** shows the histopathological findings of the excised appendices.

Early diagnosis is important to prevent complications and reduce the morbidity rate. However, preoperative diagnosis is very difficult and challenging due to non-specific clinical findings and therefore in most cases the diagnosis is made intraoperatively.

The clinical signs of acute appendicitis are generally lacking, because the appendix is isolated from the peritoneal cavity with hernia sac due to the tight neck of the femoral ring, so signs of peritonitis are rarely developed.

De Garengeot's hernia usually presents with incarcerated femoral hernia with painful, swollen and irreducible groin tumefaction, surrounding erythema, and unclear abdominal pain [14]. In laboratory findings, leukocytosis and elevated CRP may indicate an inflammatory process [25].

Plain abdominal X-ray, US, computed tomography (CT), and magnetic resonance imaging (MRI) are helpful in the diagnostic process. X-ray findings are usually nonspecific, but they can exclude obstruction of the small intestine. Imaging diagnostics

are very helpful in confirming or excluding the diagnosis of acute appendicitis in an emergency.

Ultrasonography is cheaper and more easily accessible than CT and MRI, but its disadvantage is subjective evaluation [26]. Ultrasound can identify bowel and (or) fluid contents in the hernia sac. Real-time direct US signs of acute appendicitis are non-compressive, aperistaltic tubular appendix structure arising from the base of the cecum, target lesion or presence of a bull's eye sign, appendix diameter > 6 mm, single wall thickness ≥ 3 mm, hypervascularity on color Doppler and contrast-enhanced US in the early phase or hypo-avascularity in case of abscess and necrosis. Indirect signs are increased echogenicity of local mesenteric fat, local abscess formation, free fluid surrounding the appendix, increased local mesenteric lymph nodes, thickening of the peritoneum, and signs of secondary obstruction of the small intestine [26].

Filatov et al. indicate that it is sonographically possible to describe the exact relation between hernia and the femoral vessels, which is important for preoperative diagnosis of the femoral hernia type [27]. Abdominal CT is the best diagnostic procedure for de Garengeot's hernia. In a prospective study, Gaitini et al. reported that sensitivity and specificity of CT in the diagnosis of appendicitis were 100%, 98.9%, respectively, whereas sensitivity and specificity of US were 74.2% and 97% [28]. In a meta-analysis, van Randen et al. reported that the sensitivity and specificity of CT in the diagnosis of acute appendicitis were 91% and 90%, respectively, and the sensitivity and specificity of graded compression US were 78% and 83% [29].

The CT criteria for the diagnosis of acute appendicitis are transverse diameter of the appendix greater than 6 mm, presence of an appendicolith, periappendiceal fluid, and absence of gas or intestinal contrast within the appendix [30, 31]. The CT finding of low-set cecum with tubular structure inside the hernia sac with or without liquid content and stranding of nearby fat is 98% specific and sensitive for the diagnosis of de Garengeot's hernia [32].

The MRI is only used when CT is contraindicated, because the costs are much higher. Its sensitivity and specificity in these circumstances are 97% and 95%, respectively. According to the literature from PubMed, the first description of the Garengeot's hernia using MRI was reported by Halpenny in 2012 [27, 33, 34].

In the diagnostic work-up of patients, in the analyzed studies, CT was used in 61/133 patients, US in 23/133, MRI in 2/133 and native abdominal radiography in 35/133. The accurate diagnosis of Garengeot's hernia was made by CT in 53/61 patients (86.8%), MRI in 2/2 (100%) and US in 1/23 patients (4.7%).

If de Garengeot's hernia with an inflamed appendix is not recognized or the patient is not operated urgently, serious and life-threatening complications may occur, such as abscess collection, necrotizing fasciitis, necrosis of the hernia contents with perforation and sepsis, intestinal obstruction and death [35–38].

The treatment of de Garengeot's hernia is emergency surgery, but there is no consensus on the choice of surgical approach and surgical procedure. The choice of surgical technique depends on the skill of the surgeon, complications of the hernia, comorbidities and the general condition of the patient. Surgical solutions include laparoscopic or open access with mesh or simple herniorrhaphy, with or without appendectomy. The surgical approaches described in the treatment of de Garengeot's hernias are inguinal, inguinal and laparotomy, laparotomy, laparoscopy, laparoscopy and inguinal, laparoscopy and laparotomy [32].

Open surgical approaches are Lockwood's infrainguinal (femoral), Lotheissen transinguinal, and modified McEvedy's high incisions, the de Oliveira's technique and the inguinal King's College approach [35]. Laparotomy can be done as a midline incision or as an incision in the right lower quadrant. In the last decade, there have been an increasing number of reports and supporters of the laparoscopic approach and laparoscopic appendectomy with laparoscopic mesh or non-mesh repair of femoral hernia defect.

Some authors advocate a hybrid surgery - laparoscopic approach and laparoscopic appendectomy with open mesh or non-mesh repair of de Garengeot's hernia [25, 39, 40].

However, the laparoscopic approach has a higher risk of intraperitoneal contamination when the patient has advanced clinical signs of appendicitis. In these cases, open appendectomy and non-mesh herniorrhaphy should be performed with a supra-inguinal, inguinal, transinguinal, or subinguinal approach, which are considered to be a better and safer solution. The advantage of the laparoscopic approach is prevention of surgical site infection and contamination of the mesh, though more authors recommend suture hernioplasty instead of mesh repair [36, 41].

Depending on the findings of the appendix, there are numerous surgical procedures in the treatment of de Garengeot's hernia. These include open hernia repair and appendectomy, open laparotomy repair, diagnostic laparoscopy with appendectomy and open hernia repair, initial hernia repair followed by interval appendectomy, and initial appendectomy followed by interval hernia repair [42].

The choice of surgical procedure in the treatment of de Garengeot's hernia depends on the inflammation of the appendix. There is a consensus, that repair of the femoral hernia with prosthetic mesh is safe if there is no wound infection, abscess in the hernia sac or perforation of the appendix [3]. In the analyzed studies, most patients underwent open surgery. The most common surgical approach for de Garengeot's hernia was inguinal. The laparoscopic approach to the treatment of de Garengeot's hernia is uncommon and still controversial [43].

In 2007, Comman et al. described the first simultaneous laparoscopic appendectomy with transabdominal preperitoneal mesh repair (TAPP) of de Garengeot's hernia [44]. A laparoscopic approach with appendectomy and total extraperitoneal repair

Table 2. Distribution of surgical approaches in the treatment of de Garengeot's hernia
Tabela 2. *Distribucija hirurškog pristupa u tretmanu De Garengeove hernije*

Surgical approach/*Hirurški pristup*	Number of cases/*Broj slučajeva (n)*	Percent/*Procenat (%)*
Inguinal/*Ingvinalni*	95	71.4
TAPP/*TAPP*	4	3
TEP/*TEP*	1	0.8
Laparoscopy and inguinal/*Laparoskopski i ingvinalni*	8	6
Laparoscopy and laparotomy/*Laparoskopski i laparotomija*	1	0.8
Laparotomy/*Laparotomija*	5	3.7
Inguinal and laparotomy/*Ingvinalni i laparotomija*	15	11.3
Unknown/*Nepoznat*	4	3

Legend: TAPP - transabdominal preperitoneal approach; TEP - total extraperitoneal approach
Legenda: TAPP – transabdominalni preperitonealni pristup; TEP – totalni ekstraperitonealni pristup

(TEP) of de Garengeot's hernia repair was first reported by Beysens et al. [45]. A laparoscopic approach (TAPP, TEP) was performed in 4 patients, a combination of a laparoscopic approach with an inguinal incision and femoral hernia repair in 8, and a combination of a laparoscopic approach and a laparotomy in 1 patient **(Table 2)**. In our opinion, laparoscopic repair of de Garengeot's hernia should be performed by experienced endoscopic surgeons, because of potentially dangerous surgical complications.

Femoral hernia repair was performed with mesh in 36 patients, four of whom had a plug, and in 97 patients the repair was done with a suture technique. None of the authors used mesh/plug in case of a perforated appendix.

Postoperative complications are associated with advanced age, delayed diagnosis, nutritional status of patients and treatment [32]. The infection rates were found in 29%, while severe complications such as necrotizing fasciitis and death were rare [24, 46]. In emergency femoral hernia, mortality of 4 – 11% is reported [46–48]. In the case studies presented, there was no documented mortality.

Conclusion

De Garengeot's hernia is a rare entity and a type of femoral hernia that requires immediate treatment to prevent serious complications. The diagnostic imaging procedures (ultrasound, computed tomography) should be used in diagnosis and the choice of surgical procedure should be adapted to the patient's general condition and the skills of the surgeon. In the treatment of de Garengeot's hernia, open surgery should be given priority regarding greater patient safety and taking into consideration more technical challenges and potentially more dangerous complications that may occur during laparoscopic access by an inexperienced surgeon. The laparoscopic approach is technically more challenging, but it is a safe surgical treatment of de Garengeot's hernia in centers where laparoscopy is routinely performed.

References

1. HerniaSurge Group. International guidelines for groin hernia management. Hernia. 2018;22(1):1-165.

2. Beauchamp RD, Evers BM, Mattox KL, Sabiston DC, Townsend CM Jr. Sabiston textbook of surgery. 20th ed. Philadelphia: Elsevier; 2017.

3. Suzuki S, Furui S, Okinaga K, Sakamoto T, Murata J, Furukawa A, et al. Differentiation of femoral versus inguinal hernia: CT findings. AJR Am J Roentgenol. 2007;189(2):W78-83.

4. Kalles V, Mekras A, Mekras D, Papapanagiotou I, Al-Harethee W, Sotiropoulos G, et al. De Garengeot's hernia: a comprehensive review. Hernia. 2013;17(2):177-82.

5. Bay-Nielsen M, Kehlet H, Strand L, Malmstrøm J, Andersen FH, Wara P, et al. Quality assessment of 26,304 herniorrhaphies in Denmark: a prospective nationwide study. Lancet. 2001;358(9288):1124-8.

6. Arenal JJ, Rodríguez-Vielba P, Gallo E, Tinoco C. Hernias of the abdominal wall in patients over the age of 70 years. Eur J Surg. 2002;168(8-9):460-3.

7. Guenther TM, Theodorou CM, Grace NL, Rinderknecht TN, Wiedeman JE. De Garengeot hernia: a systematic review. Surg Endosc. In press. Doi: https://doi.org/10.1007/s00464-020-07934-5.

8. Brown N, Moesbergen T, Steinke K. The French and their hernias: prospective radiological differentiation of de Garengeot from other groin hernias. J Radiol Case Rep. 2013;7(4):16-21.

9. De Luca GM, Franzoso L, Peerrone A, Tromba A, Zoubi AA, De Luca A, et al. De Garengeot's hernia with acute appendicitis. Case report and literature review. New Dac classification. International Journal of Recent Scientific Research. 2019;10(12C):36425-8.

10. De Garengeot RJC. Traite des operations de chirurgie. 2nd ed. Paris: Huart; 1731. p. 369-71.

11. Akopian G, Alexander M. De Garengeot hernia. Appendicitis within a femoral hernia. Am Surg. 2005;71(6):526-7.

12. Ivanschuk G, Cesmebasi A, Sorenson EP, Blaak C, Loukas M, Tubbs SR. Amyand's hernia: a review. Med Sci Monit. 2014;20:140-6.

13. Patoulias D, Kalogirou M, Patoulias I. Amyand's hernia: an up-to-date review of the literature. Acta Medica (Hradec Kralove). 2017;60(3):131-4.

14. D'Alia C, Lo Schiavo MG, Tonante A, Taranto F, Gagliano E, Bonanno L, et al. Amyand's hernia: case report and review of the literature. Hernia. 2003;7(2):89-91.

15. Konofaos P, Spartalis E, Smirnis A, Kontzoglou K, Kouraklis G. De Garengeot's hernia in a 60-year-old woman: a case report. J Med Case Rep. 2011;5:258.

16 Nguyen ET, Komenaka IK. Strangulated femoral hernia containing a perforated appendix. Can J Surg. 2004;47(1):68-9.

17. Zissin R, Brautbar O, Shapiro-Feinberg M. CT diagnosis of acute appendicitis in a femoral hernia. Br J Radiol. 2000;73(873):1013-4.

18. Schumpelick V, Dreuw B, Ophoff K, Prescher A. Appendix and cecum. Embryology, anatomy, and surgical applications. Surg Clin North Am. 2000;80(1):295-318.

19. Ahmed I, Asgeirsson KS, Beckingham IJ, Lobo DN. The position of the vermiform appendix at laparoscopy. Surg Radiol Anat. 2007:29(2):165-8.

20. Garcia-Amador C, De la Plaza R, Arteaga V, Lopez-Marcano A, Ramia J. Garengeot's hernia: two case reports with CT diagnosis and literature review. Open Med (Wars). 2016;11(1):354-60.

21. 722. Akbari K, Wood C, Hammad A, Middleton S. De Garengeot's hernia: our experience of three cases and literature review. BMJ Case Rep. 2014. Doi:10.1136/bcr-2014-205031.

23. Barbaros U, Asoglu O, Seven R, Kalayci M. Appendicitis in incarcerated femoral hernia. Hernia. 2004;8(3):281-2.

24. Thomas B, Thomas M, McVay B, Chivate J. De Garengeot hernia. JSLS. 2009;13(3):455-7.

25. Sharma H, Jha PK, Shekhawat NS, Memon B, Memon MA. De Garengeot hernia: an analysis of our experience. Hernia. 2007;11(3):235-8.

26. Mostbeck G, Adam EJ, Nielsen MB, Claudon M, Clevert D, Nicolau C, et al. How to diagnose acute appendicitis: ultrasound first. Insights Imaging. 2016;7(2):255-63.

27. Filatov J, Ilibitzki A, Davidovitch S, Soudack M. Appendicitis within a femoral hernia: sonographic appearance. J Ultrasound Med. 2006;25(9):1233-5.

28. Gaitini D, Beck-Razi N, Mor-Yosef D, Fischer D, Ben Itzhak O, Krausz MM, et al. Diagnosing acute appendicitis in adults: accuracy of color Doppler sonography and MDCT compared with surgery and clinical follow-up. AJR Am J Roentgenol. 2008;190(5):1300-6.

29. Van Randen A, Bipat S, Zwinderman AH, Ubbink DT, Stoker J, Boermeester MA. Acute appendicitis: meta-analysis of diagnostic performance of CT and graded compression US related to prevalence of disease. Radiology. 2008;249(1):97-106.

30. Ishiyama M, Yanase F, Taketa T, Makidono A, Suzuki K, Omata F, et al. Significance of size and location of appendicoliths as exacerbating factor of acute appendicitis. Emerg Radiol. 2013;20(2):125-30.

31. Yeung KW, Chang MS, Hsiao CP. Evaluation of perforated and nonperforated appendicitis with CT. Clin Imaging. 2004;28(6):422-7.

32. Kagan Coskun A, Kilbas Z, Yigit T, Simsek A, Harlak A. De Garengeot's hernia: the importance of early diagnosis and its complications. Hernia. 2012;16(6):731-3.

33. Barger RL Jr, Nandalur KR. Diagnostic performance of magnetic resonance imaging in the detection of appendicitis in adults: a meta-analysis. Acad Radiol. 2010;17(10):1211-6.

34. Halpenny D, Barrett R, O'Callaghan K, Eltayeb O, Torreggiani WC. The MRI findings of a de Garengeot hernia. Br J Radiol. 2012;85(1011):e59-61.

35. Pitchaimuthu M, Dace S. A rare presentation of appendicitis as groin swelling: a case report. Cases J. 2009;2(1):53.

36. Mizumoto R, Hendahewa R, Premaratne G. De Garengeot hernia-use of a novel surgical approach and literature review. Int J Surg Case Rep. 2016;19:127-30.

37. Voitk AJ, MacFarlane JK, Estrada RL. Ruptured appendicitis in femoral hernias: report of two cases and review of the literature. Ann Surg. 1974;179(1):24-6.

38. Wyatt JP, Varma JS. Femoral hernia appendix causing small intestinal obstruction. Postgrad Med J. 1992;68(797):223-4.

39. Ramsingh J, Ali A, Cameron C, Al-Ani A, Hodnett R, Chorushyj C. De Garengeot's hernia: diagnosis and surgical management of a rare type of femoral hernia. J Surg Case Rep. 2014;2014(2).

40. Al-Subaie S, Mustafa H, Al-Sharqawi N, Al-Haddad M, Othman F. A case of de Garengeot hernia: the feasibility of laparoscopic transabdominal preperitoneal hernia repair. Int J Surg Case Rep. 2015;16:73-6.

41. Shiihara M, Kato T, Kaneko Y, Yoshitoshi K, Ota T. De Garengeot hernia with appendicitis treated by two-way-approach surgery: a case report. J Surg Case Rep. 2017;2017(7):rjx140.

42. Granvall SA. De Garengeot hernia: a unique surgical finding. JAAPA. 2014;27(5):39-41.

43. Talini C, Oliveira LO, Araújo AC, Netto FA, Westphalen AP. De Garengeot hernia: case report and review. Int J Surg Case Rep. 2015;8C:35-7.

44. Comman A, Gaetzschmann P, Hanner T, Behrend M. DeGarengeot hernia: transabdominal preperitoneal hernia repair and appendectomy. JSLS. 2007;11(4):496-501.

45. Beysens M, Haeck L, Vindevoghel K. Laparoscopic appendectomy combined with TEP for de Garengeot hernia: case report. Acta Chir Belg. 2013;113(6):468-70.

46. Mashima H, Banshodani M, Nishihara M, Nambu J, Kawaguchi Y, Shimamoto F. De Garengeot hernia with perforated appendicitis and a groin subcutaneous abscess: a case report. Int J Surg Case Rep. 2017;33:8-11.

47. Dahlstrand U, Wollert S, Nordin P, Sandblom G, Gunnarsson U. Emergency femoral hernia repair: a study based on a national register. Ann Surg. 2009;249(4):672-6.

48. Lundstrom KJ, Sandblom G, Smedberg S, Nordin P. Risk factors for complications in groin hernia surgery: a national register study. Ann Surg. 2012;255(4):784-8.

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CONGENITAL ANOMALIES OF THE EPIDIDYMIS

KONGENITALNE ANOMALIJE EPIDIDIMISA

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Summary

Introduction. Congenital anomalies of the epididymis, detected either during orchiopexy or routine herniectomy, as well as their importance in male fertility, have been described in many papers in clinical surgery. **Clinical Considerations.** The aim of this study was to report various types of epididymal congenital abnormalities registered in the clinical practice of pediatric surgery, to describe their basic characteristics and to classify them into three groups: (I) fusional anomalies of the epididymis and testis; (II) anatomic forms of anomalies; (III) congenital epididymal cysts. **Conclusion.** The clinical experience of pediatric surgeons indicates that during these surgical procedures, it is necessary to carefully examine the epididymis to detect anomalies, and it is of utmost importance to inform the patient and/or his parents. It is also recommended to follow the patients up to the age of fertility.

Key words: Congenital Abnormalities; Epididymis; Orchiopexy; Cryptorchidism; Diagnosis; Fertility; Child

Sažetak

Uvod. Kongeninalne anomalije epididimisa otkrivene tokom orhidopeksije ili herniektomije kao i njihov značaj u fertilnoj funkciji muškarca, opisani su u mnogim radovima kliničke hirurgije. **Klinička razmatranja**. Cilj rada je da se dokumentuju različiti oblici kongenitalnih anomalija epididimisa koji su registrovani u kliničkoj praksi dečjih hirurga, da se opišu njihove osnovne karakteristike i da se napravi klasifikacija anomalija u tri grupe: 1) anomalije fuzije epididimisa sa testisom, 2) anomalije anatomske forme i 3) cistične promene epididimisa. **Zaključak.** Kliničko iskustvo dečjih hirurga ukazuje na to da je tokom navedenih operacija neophodno pažljivo pregledanje epididimisa kako bi se uočile anomalije i da je neophodno o tome upoznati pacijenta i/ili njegove roditelje. Savetuje se praćenje pacijenta do njegovog fertilnog perioda života.

Ključne reči: kongenitalne anomalije; epididimis; orhidopeksija; kriptorhidizam; dijagnoza; fertilitet; dete

„In a such delicate and complex tubular system as that of the testis, epididymis and vas deferens it is not suprising that failure of conduction sometimes occur."
Scorer & Farrington, 1971

Introduction

Over the last few decades, due to the rise of public interest in male infertility, during inguinal surgery surgeons payed more attention to epididymal abnormalities [1, 2]. As reported by many authors, the incidence of epididymal abnormalities in boys with undescended testis [3, 4] and the significance of testicular location are the point of interest: the higher the arrest of testicular descent, the more grossly abnormal is the associated ductal system [5]. Regarding the incidence of abnomalities, a comparison between the group of patients with undescended testis and group having widely patent processus vaginalis, has been drawn [3, 5]. The pathogenesis of epididymal abnormalities is still not fully elucidated, but it could be a part of a wide spectrum of embryopathic conditions. We have reviewed various epididymal anomalies encountered during different types of inguinal surgical procedures.

Clinical Considerations

A normal epididymis consists of head, body and tail. The sperm tubular system begins with rete testis and seminiferous tubules, efferent ducts, epididymal duct and vas deferens, and continues to urethra that empties outside the body [6]. Epididymis is responsible for: 1. transporting the sperm by peristaltic contractions, and 2. sperm to acquire motility and ability to fertilize the ovum [7]. Undisturbed sperm transport is very important for male fertility and detection of possible discontinuity through this tubular system is mandatory [6].

During the embryonic development, some abnormalities may occur. Different origin of upper and

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Abbreviations
TE – testis epididymis
EC – epididymal cysts

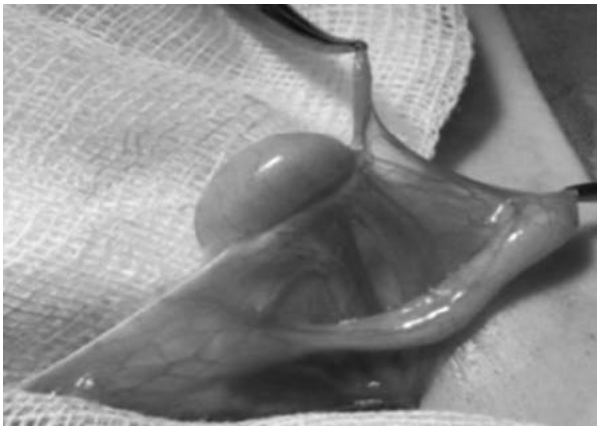

Figure 1. Loss of continuity between the testis and epididymis
Slika 1. *Ne postoji povezanost testisa i epidimisa*

distal parts of epididymis, and their point of union are of interest regarding epididymal maldevelopment that may result in congenital abnormalities [3, 5, 8].

These congenital anomalies may be classified as follows:

I. Abnormalities of testis – testits epididymis (TE) fusion
II. Abnormalities of anatomic forms
III. Epididymal cysts (EC)

Abnormalities of testis - epidymal fusion

Firm attachment between the rete testis and the caput of epididymis by efferent ducts is necessary for sperm passage [6]. Sometimes, there is a few millimeter gap between testis and the epididymal head and in such patients the TE conduction is discussable **(Figure 1).** If the gap is long, the TE continuity is not present and sperm transport is obstructed [9, 10].

Abnormalities of anatomic forms

Embryologically, the testis and epididymis have two origins: gonads and the upper part of epididymis derive from the genital ridge, but distal part of the epididymis and vas deferens from the mesonephric duct [4, 6]. The point of union is important for the continuity of ductal system. Developmental abnormalities may be: completely absent, agenesis of the epididymis **(Figure 2)**; partly undeveloped, hypoplastic head when the histology structures are maldeveloped; blind ended at the epididymal tail with no connection with vas deferens. The absence of the distal part of epididymis **(Figure 3)** and of vas deferens, noticed during inguinal surgery, indicates further investigation of embryogenesis defects that could include ispilateral renal agenesis [11, 12]. At the point of union, complete atresia is possible or some kind of a constriction **(Figure 4)**. Also, the epididymis may be angled at the midpoint **(Figure 5).**

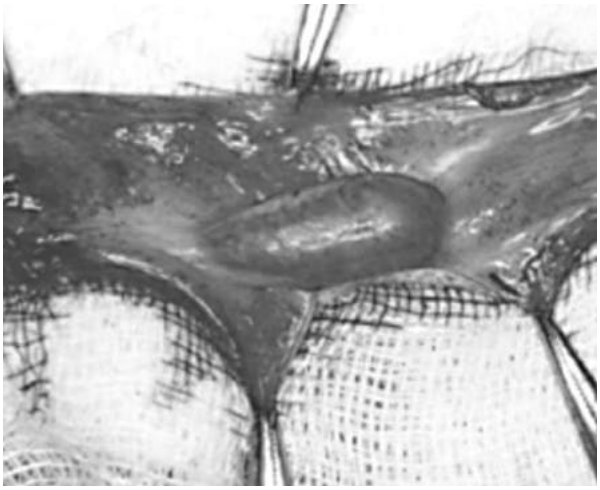

Figure 2. Agenesis of epididymis - complete absence of epididymis
Slika 2. *Agenezija epididimisa – epididimis u celini nedostaje*

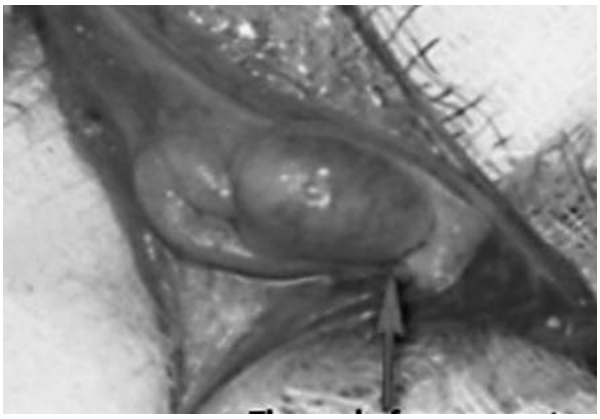

Figure 3. Upper part of epididymis is present, distal part is absent. The vas deferens is present as a fine thread. The follow up study revealed an ipsilateral renal agenesia
Slika 3. *Postoji gornja polovina epididimisa, distalni deo nedostaje. Vas deferens je prisutan samo kao končasta nit. Kliničko ispitivanje je pokazalo da postoji istostrana renalna agenezija*

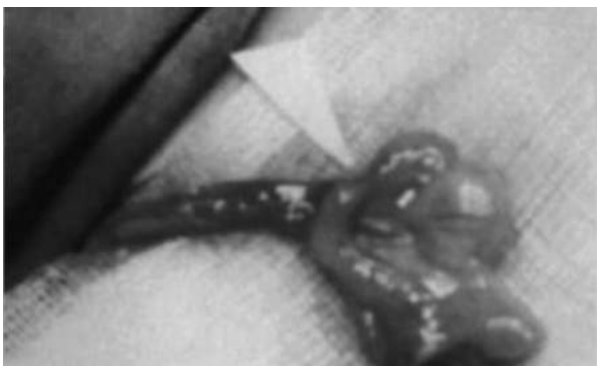

Figure 4. The white arrow points to the narrowed section
Slika 4. *Strelica pokazuje suženje u srednjem delu epididimisa*

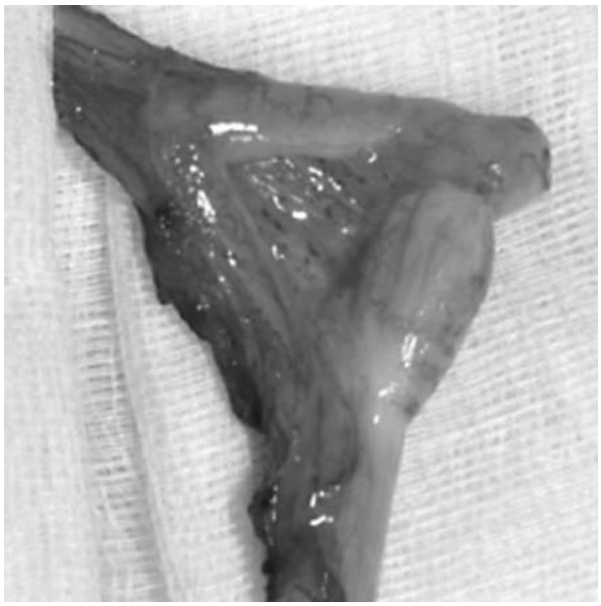

Figure 5. Epididymis is sharply angled at about the midpoint
Slika 5. *Epididimis pokazuje oštar ugao u srednjem delu*

The normal length of epididymis is one and a half of testicular length. Sometimes the length of epididymis is two, three or more than the normal. The role of the angled and long-loop epididymis in the male fertile is still not clear, but impairment in sperm maturation and hindered transportation can be expected [13].

Epididymal Cysts

Epididymal cysts are round and smooth cystic masses that occur usually in the head of epididymis. They are rare in children and are mostly observed during puberty and adulthood. The etiology is unknown, but the proposed theories include dilated efferent ducts that have failed to fuse with epididymis [14, 15] and/or part of a testicular dysgenetic syndrome caused by an endocrine abnormality [16, 17]. The cysts are often asymptomatic and benign.

In adolescents, EC usually presents a painless scrotal swelling **(Figure 6)** as a result of dilatation of the efferent epididymal tubules and does not contain sperm [17, 18]. Ultrasonography imaging expertise is necessary to exclude testicular tumors and other associated diseases. Sonographically, they are thin-walled, simple rare septated cysts, located within the epididymal head [18] with appropriate echoes. Many cases (up to 60%) regress spontaneously [19]. The average time to involute ranges from 4 to 50 months. However, a conservative approach is recommended in children [16], but periodic ultrasound follow-up is suggested in the majority, especially in pediatric cases. A conservative management is suggested for cysts smaller than 10 mm [17, 18]. Surgery is recommended in some patients, due to no manageable testicular pain or increased paratesticular mass of cysts over 10 mm in diameter. Although an author [17] reported a risk of recurrence after surgery, in our series as well as in the other authors, no recurrences have been found. Torsion of the cyst has also been described [19, 20].

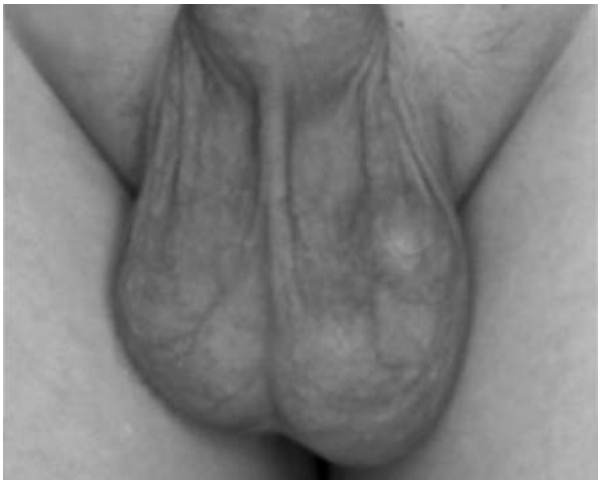

Figure 6. A boy at the beginning of puberty self-palpated a mass within the left hemiscrotum
Slika 6. *Dečak na početku puberteta samopregledom je napipao formaciju u predelu leve polovine skrotuma*

Survey of clinical cysts presentation:
- solitary cyst, usually within the head
- multicystic epididymis [1]
- completely cystic epididymis.

Conclusion

Although orchidopexy performed in young children with undescended testis may prevent infertility, associated anomalies of the epididymis and vas deferens may affect the result of surgery.

The authors stress the necessity of meticulous examination of the testis, epididymis and vas deferens during inguinal exploration and consider all anomalies found as factors that may compromise fertility.

Careful inspection during orchidopexy or any inguinoscrotal surgery can identify the location of the obstruction. If the abnormalities are noticed bilaterally, the problem is more difficult pointing to obstructive infertility. Also, multiple abnormalities along the sperm duct system or combined with other genital or urinary organs have to be dealt with. Counseling the patient's parents and/or patients is necessary to make a decision on proper treatment for natural fertility. In order to identify the exact clinical significance of a wide spectrum of epididymal abnormalities, long-term data are necessary.

References

1. Scorer CG, Farrington GH. Congenital deformities of the testis and epididymis. New York: Appleton-Century-Crofts; 1971. p. 136-46.

2. Mollaeian M, Mehrabi V, Elahi B. Significance of epididymal and ductal anomalies associated with undescended testis: study in 652 cases. Urology. 1994;43(6):857-60.

3. Caterino S, Lorenzon L, Cavallini M, Cavaniglia D, Ferro F. Epididymal-testicular fusion anomalies in cryptorchidism are associated with proximal location of the undescended testis and with a widely patent processus vaginalis. J Anat. 2014;225(4):473-8.

4. Baku Fahmy MA. Rare congenital genitourinary anomalies. Berlin: Springer; 2015. p. 77-80.

5. Barthold JS, Redman JF. Association of epididymal anomalies with patent processus vaginalis in hernia, hydrocele and cryptorchidism. J Urol. 1996;156(6):2054-6.

6. Robaire B, Hinton BT. The epididymis. In: Plant TM, Zeleznik AJ, editors. Knobil and Neill's physiology of reproduction. 4th ed. Amsterdam: Elsevier; 2015. p. 691-771.

7. DePalma L, Carter D, Weiss RM. Epididymal and vas deferens immaturity in cryptorchidism. J Urol. 1988;140(5 Pt 2):1194-6.

8. Arroteia KF, Garcia PV, Barbieri MF, Justino ML, Pereira LAV. The epididymis: embryology, structure, function and its role in fertilization and infertility. In: Pereira LAV, editor. Embryology - updates and highlight on classic topics. Rijeka: InTech; 2012. p. 41-66.

9. Merksz M. Fusional anomalies of the testis and epididymis. Acta Chir Hung. 1998;37(3-4):153-70.

10. Dean Al Jr, Major JW, Ottenheimer EJ. Failure of fusion of the testis and epididymis. J Urol. 1952;68(4):754-8.

11. Pichler R, Oswald J, Glodny B, Skradski V, Aigner F, Rehder R. Unilateral renal agenesis with absent ductus deferens, epididymis and seminal vesicle: incidental finding in a 22-year-old patient with maldevelopment of the mesonephric duct. Urol Int. 2011;86(3):365-9.

12. Nikam V, Nagure P, Patil P. Unilateral left renal agenesis associated with congenital agenesis of vas deferens and seminal vesicle: a case report. International Journal of Medical Research and Health Sciences. 2018;7(5):83-7.

13. Koff WJ, Scaletscky R. Malformations of the epididymis in undescended testis. J Urol. 1990;143(2):340-3.

14. Vohra S, Morgentaler A. Congenital anomalies of the vas deferens, epididymis and seminal vesicles. Urology. 1997; 49(3):313-21.

15. Comiter CV, Bruning CO 3rd, Morgentaler A. Detached ciliary tufts in the epididymis: a lesson in applied anatomy. Urology. 1995;46(5):740-2.

16. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972-8.

17. Homayoon K, Suhre CD, Steinhardt GF. Epididymal cysts in children: natural history. J Urol. 2004;171(3):1274-6.

18. Erikci V, Hoşgör M, Aksoy N, Okur Ö, Yildiz M, Dursun A, et al. Management of epididymal cysts in childhood. J Pediatr Surg. 2013;48(10):2153-6.

19. Bleve C, Conighi ML, Bucci V, Costa L, Chiarenza SF. Torsion of huge epididymal cyst in a 16-year-old boy: case report and review of the literature. Pediatr Med Chir. 2018;40(1):162, 20-2.

20. Karaman A, Afsarlar CE, Arda N. Epididymal cyst: not always a benign condition. Int J Urol. 2013;20(94):457-8.

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MEDICAL AND TECHNICAL ASPECTS OF ACTIVITIES AT HOSPITAL DEPARTMENTS OF INFECTIOUS DISEASES DURING THE PANDEMIC OF THE CORONAVIRUS DISEASE 2019 AND OTHER HIGHLY INFECTIOUS DISEASES

MEDICINSKI I TEHNIČKI ASPEKTI AKTIVNOSTI U BOLNIČKIM ODELJENJIMA ZA INFEKTIVNE BOLESTI TOKOM PANDEMIJE VIRUSOM CORONA 2019 I DRUGIM TEŠKIM INFEKTIVNIM BOLESTIMA

Elena ALEKSANDROVNA BOENKO[1], Leonid ANDREEVICH REPIN[2] and Lyudmila LEONIDOVNA REPINA[3]

Summary

Introduction. The article presents information on the activities of inpatient facilities for infectious diseases during the pandemic of the new coronavirus disease 2019, taking into account current regulatory documents. The authors reviewed the principles of hospital zoning and patient controlled movement in hospitals for infectious diseases. The paper deals with the organization of the admission departments and equipment of the diagnostic units of hospitals for infectious diseases, providing a graphical presentation of an individual isolation unit (Melzer box) including description of the control and management system for safe access to infectious units. The characteristics of engineering and communication systems, disposal of hazardous medical waste, catering, navigation systems, operational remote communication between doctors and patients, application of barcoding for patient identification and medical records are also discussed. The purpose of this paper was to: Identify the shortcomings of the existing regulatory framework concerning the management and organization of health care institutions that provide medical care to patients with the new coronavirus infection; Identify areas that require adjustments, given the modern requirements for high quality treatment, as well as to ensure epidemiological safety for medical staff and patients; Specify additional requirements for hospitals for infectious diseases, which should be taken into account when planning major repairs, reconstruction and construction of new medical facilities for providing health care during the pandemic of new coronavirus disease 2019 and other highly infectious infections.

Key words: Pandemics; Coronavirus Infections; Documentation; Infection Control; Hospital Units; Clinical Protocols; Hospitalization; Patient Admission; Russia; Disaster Planning

Sažetak

Uvod. U članku su predstavljene informacije o aktivnostima jedinica u bolnicama za zarazne bolesti tokom pandemije nove infekcije korona virusom (COVID-19), uzimajući u obzir trenutne dokumente koji te aktivnosti regulišu. Autori su pregledali principe zoniranja prostorija i usmeravanja pacijenata u infektivnoj bolnici. Predstavljene su informacije o karakteristikama organizacije prijemnog odeljenja i opremanja dijagnostičkih jedinica bolnice za infektivne bolesti; označene su šeme uređaja i funkcionisanja Melzerovih kutija. Dat je opis sistema kontrole i upravljanja sigurnim pristupom jedinicama zarazne bolnice. Navedene su karakteristike inženjerskih sistema i komunikacija u bolnicama za zarazne bolesti, odlaganje opasnog medicinskog otpada, organizacija ishrane, navigacioni sistemi, operativna komunikacija na daljinu između lekara i pacijenata, primena bar kodiranja za identifikaciju pacijenata i medicinsku dokumentaciju. Cilj rada bio je identifikovati nesavršenost postojećih dokumenata koji regulišu aranžman i organizaciju aktivnosti zdravstvenih ustanova koje pružaju medicinsku negu pacijentima sa novom infekcijom korona virusom (COVID-19); odrediti oblasti koje zahtevaju prilagođavanja, c obzirom na savremenim zahtevima kvaliteta lečenja, kao i da obezbedi epidemiološke bezbednosti medicinskog osoblja i pacijenata, označiti dodatne zahteve za bolnice za zarazne bolesti, koje je prikladno uzeti u obzir prilikom pripreme medicinsko-tehničkih zadataka za velike popravke, rekonstrukcije i novu izgradnju medicinskih objekata za pružanje zdravstvene zaštite tokom pandemije nove infekcije korona virusom (COVID-19) i drugih posebno opasnih infekcija.

Ključne reči: pandemija; koronavirus infekcije; dokumentacija; kontrola infekcije; bolničke jedinice; bolnički protokoli; hospitalizacija; prijem pacijenata; Rusija; plan za vanredne situacije

Introduction

The activities of medical facilities rendering health care services for patients with the new coronavirus disease 2019 (COVID-19), which was ranked as a highly infectious disease by the World Health Organization, have revealed the deficiencies of the regulatory documents in force which have been used when projecting and constructing hospitals and departments for infectious diseases in Russia. In order to increase the

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Abbreviations
COVID-19 – coronavirus disease 2019
ACS – access control system
UV – ultraviolet

quality and safety of medical treatment, prevent cross colonization, ensure the epidemiological safety of medical personnel and prevent infections connected with rendering medical care, in this article we propose some additional requirements for in-patient facilities for infectious diseases which should be taken into account when preparing medical and technical terms of reference for conducting major repairs, reconstruction and construction of new medical facilities for medical care of patients with infectious diseases.

Admission office of inpatient facilities for infectious diseases

The admission office of hospitals for infectious diseases must be equipped with separate isolation wards for admission and discharge of patients. Taking into account mass admission of patients during an epidemic, it is necessary to set up isolation wards corresponding to 5% of the bed capacity of the inpatient facility. This should optimize the waiting time of patients before being admitted and hospitalized, as well as exclude lengthy delays during admission to departments for infectious diseases.

When designing an inpatient facility for infectious diseases, the project should take into account the work of the admission office in accordance with the principles of medical triage.

Under the circumstances of the COVID-19 pandemic, the practice of rendering emergency secondary care inevitably changes. In connection with the above, the issue whether a hospital for infectious diseases has an operating theatre equipped to carry out surgeries of patients with COVID-19, becomes highly relevant.

If patients with COVID-19 hospitalized at emergency departments of secondary surgery care need surgery, a special team of doctors from another medical facility should be called [1].

Diagnostic subdivisions and services

The requirements in respect to instrumental and laboratory diagnostics should be extended. In our opinion, an inpatient facility for infectious diseases needs the following medical equipment:
– Computed tomography (CT) scanner;
– X-Ray apparatus;
– Polymerase chain reaction (PCR) laboratory;
– Virology laboratory.

Premises of an inpatient facility for infectious diseases

The premises of an inpatient facility for infectious diseases must be clearly divided into three zones: red, yellow and green.

After the exit from the red zone, in the entrance hall between the red and yellow zones, it is necessary to provide a gallery-gate for medical personnel to pass through and have their protective clothes disinfected by aerosol spraying. The doors in the gallery-gate must be sliding with an electromechanical drive. The exit door from the gallery-gate to the yellow zone must open only upon completing the disinfection procedure. It is possible to use sliding transparent banded polyvinylchloride curtains closing automatically. Right after the exit from the gallery-gate, there should be a sanitary inspection room for taking off the "infected clothes" which is organized as a gate: if the entrance door is open, the second door – to exit to the clear zone – will be closed. This will stop the air moving between the premises and the infectious agents spreading through the inpatient facility.

It is recommended that the inpatient facility for infectious diseases should have at least 30% of the total bed capacity in the intensive care.

The nurses' area(s) in the intensive care department must be isolated from the intensive-care ward by a small glass isolation ward or a glass lounge in the hall.

The central monitoring system designated for centralized monitoring of patients' vital signs should be in the nurses' areas in the intensive care departments and intensive care wards. The information from bedside medical monitors should be transferred to the central monitoring system and duplicated to monitors located at doctors' rooms and centralized patient surveillance offices in real time mode. This provides the medical personnel with audio and visual notifications about any deviations of physiological parameters of a patient, as well as about technical alert signals if the monitoring system itself has an error or a failure.

The inpatient facility for infectious diseases must have the equipment for extracorporeal hemocorrection (blood purification).

Individual isolation units

Since the inpatient facility for infectious diseases provides not only medical treatment but also a secure isolation of patients in order to prevent disease dissemination, the main requirement is to protect both patients and medical personnel from nosocomial transmission.

Patients with cross infections, unclear diagnosis or highly infectious diseases should immediately be placed to individual units. The structure of an individual isolation unit is illustrated in the **Figure 1**.

In accordance with the Order of the Ministry of Health Care and Social Development of the Russian Federation No. 69n of January 31, 2012: «On Approving the Procedure for Rendering Medical Assistance to Adult Patients Having Infectious Diseases» (annex 4, clause 4) [3], a department of infectious diseases must have at least 50% individual isolation units of the total bed capacity. However, the organization of hospital space in the circum-

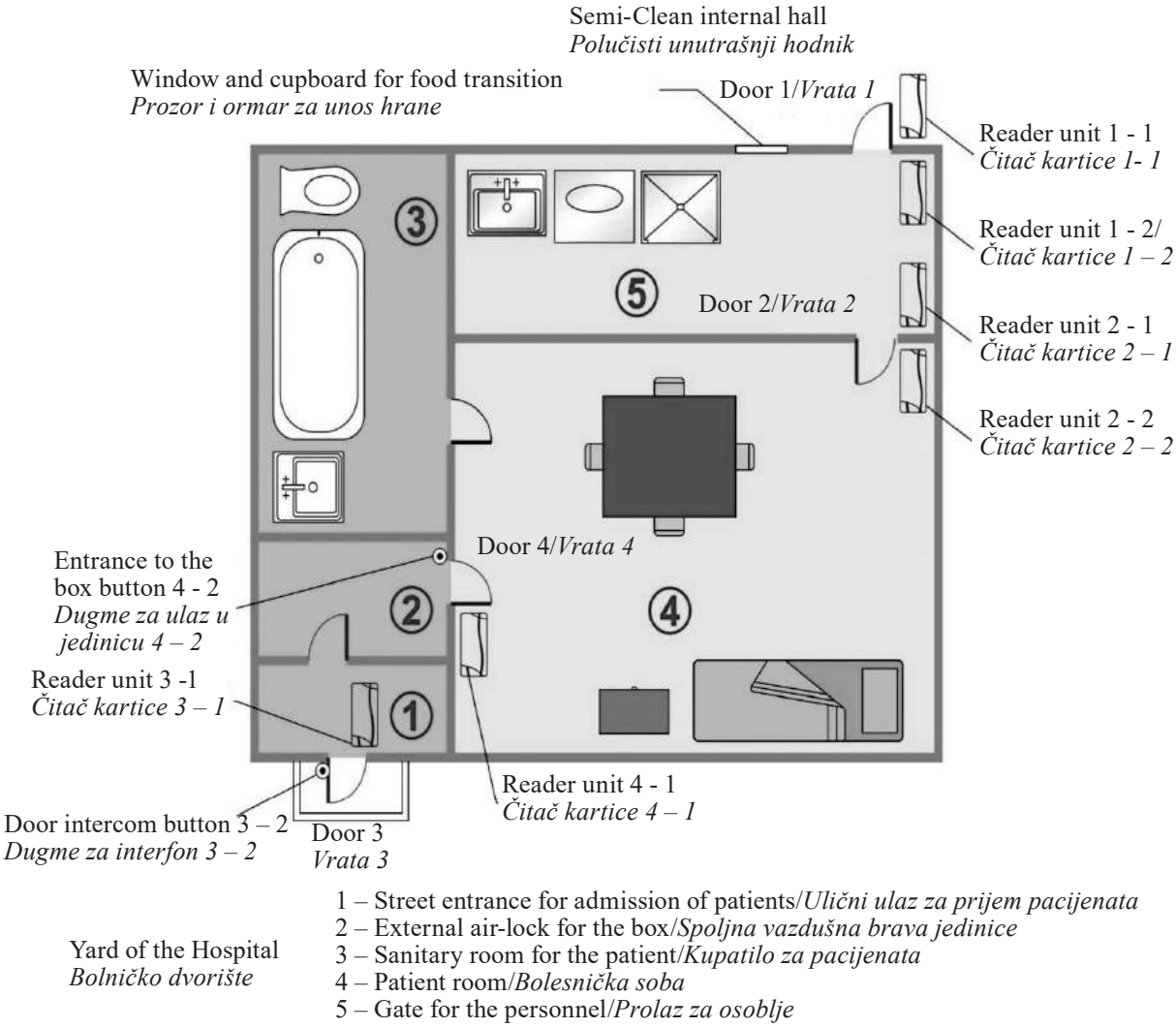

Figure 1. Structure of an individual isolation unit [2]
Slika 1. *Struktura individualne izolacione jedinice [2]*

stances of the new COVID-19 showed that the need for individual isolation units has increased to 80% of the total bed capacity [4].

It is recommended to set up electronic locks with chipped electronic cards readers, access control system (ACS) on the individual isolation unit doors leading from the hall of the inpatient facility as well as on the external door, leading from the yard of the inpatient facility for infectious diseases to the individual isolation unit. Therewith, fire safety should be provided for emergency opening of electronic locks via the ASC in case of a fire alarm.

Passage in the individual isolation units

In order to prevent air movement from one premise to another, each individual isolation unit must be provided by a safe passage for the delivery of medicines, food for patients and removal of used dishes.

The passage should include:

– Operating console with an indication system;
– System for indicating and blocking simultaneous door opening as well as blocking both doors during the work of an ultraviolet (UV) lamp;
– UV-lamp set up inside the unit. The UV-lamp is turned on by the medical personnel from the operating console and has the working regime of 5 - 6 minutes and automatic turn off. When the UV-lamp is working, both doors are blocked;
– Intercom loudspeaker in the unit, a full duplex and activation indicator;

The passage doors must have glazing and two compaction circuits. The walls inside the passage must have smooth surfaces preventing spots of light.

Engineering systems

During the construction or major repairs of inpatient facilities for infectious diseases, it is important to pay attention to modern requirements of the

engineering systems of the building, ensuring their further safe and efficient use.

Engineering systems (medical gas system, air ventilation system, air conditioning system, power supply system, heat supply system, water supply system, water disposal system and sewerage system) play the main role in sustaining the functionality of inpatient facilities for infectious diseases.

The above mentioned engineering systems allow maintaining comfortable conditions in the building, depending on the climate index and other indicators, to save physical resources.

This being said, the medical and technical component of engineering systems is of significant importance to ensure the requirements of anti-epidemic regime in inpatient facilities for infectious diseases, which is especially actual in the circumstances of the COVID-19 pandemic and other highly infectious diseases.

In order to use the engineering equipment efficiently, it is beneficial to use building computer-aided operating systems (BCAOS) or building automation and management systems (BAMS). Automating the management of these systems allows increasing their safety and security, reducing the level of risk of unauthorized switching off the engineering equipment as well as increasing the level of accident-free operation due to minimal influence of "human factor".

Medical gas system

The use of medical gases has become highly relevant when rendering medical assistance to the patients with pulmonary involvement due to the COVID-19 and other respiratory infections. Therefore, all wards should be centrally supplied with:

– Oxygen, vacuum and compressed air;

– Each bed equipped with medical gas system in intensive care departments and intensive care units should additionally be supplied with bottled helium gas.

A list of equipment of an inpatient facility for infectious diseases must include a power generator or a portable facility producing nitrogen oxide [5, 6], a facility producing thermal helium. This is necessary for successful practical application of the efficient innovative medical therapy to create a body hypoxia using helium or nitrogen oxide (working experience using this method has been accumulated in the N. V. Sklifosovsky Research Institute for Emergency Medicine) [7].

To guarantee safe supply of oxygen to patients, the central oxygen supply system must contain three independent sources of oxygen supply – primary, secondary and a reserve one. It is best to use oxygen and gasification station for the primary source, oxygen point with 40 liter oxygen balloons placed on a ramp – for the secondary source, and an oxygen generator (concentrator) – for a reserve source.

The nurse area on each floor must be equipped with a panel with sensor display of monitoring and alarm systems (with light and sound alarm) in case of sudden loss of gas supply and control of compression level in the medical gas pipeline systems.

Hyperbaric oxygenation therapy is an efficient method in the treatment of coronavirus and it was efficiently used in the N. V. Sklifosovsky [8] Research Institute for Emergency Medicine and the A. I. Burnazyan Federal Medical Biophysical Center at the Federal Medical and Biological Agency of Russia [9].

The method is based on ventilation in an altitude chamber; the oxygen is delivered to the blood at high atmospheric pressure. The blood is ventilated much more intensively than when breathing normally. It has been noted that this method of non-invasive breathing support is more efficient than delivering oxygen through a mask or high-flow oxygen [10].

In accordance with Sectoral Methodology Guidelines (OMU 42-21-27-88) [11], in the process of medical treatment, the room needs to be equipped for hyperbaric oxygenation therapy, which is reasonable to be located in the red zone.

Air handling system

It is of great importance for hospitals for infectious diseases to have a high-quality air handling system. Airborne infections cause about 90% of global infectious diseases. Therefore, the air handling systems in inpatient facilities for infectious diseases play the key role in preventing viral shedding [12].

Ventilation systems refer to systems which transmit large volumes of air with certain bacterial, viral and physical factors. An air handling system of a low quality seriously endangers the medical personnel as well as the patients.

The World Health Organization (WHO) provided rather detailed recommendations for designing air handling systems. The premises of an inpatient facility for infectious diseases, where the patients are located, must have negative pressure, door closing system, and a separate zone with independent air conditioning system. Also, it is necessary to ensure efficient mechanical aeration so that infected aerosol is removed from wards and units as soon as possible.

The negative pressure must be equal to approximately 8 – 10%. The value of the negative pressure must always be controlled via differential electronic manometers that alert the automated system of ventilation systems in case of operating trouble of the ventilation system. An alert is also made for the ward or unit entrance doors if they are not closed for more than 2 minutes.

In the wards and inpatient units for infectious diseases, it is recommended to use ventilation circuits to prevent the risk of spreading pathogenic microbes into unprotected areas. Air intake should be carried out outside, heat treated and supplied to the room. In this case, the exhaust air is taken from the room and passed through a system of fine filters of the H11-H14 type, ensuring capture of 98 – 99% of particles of 3 microns in diameter, and only after that it is discharged.

It is unacceptable to combine exhaust ventilation ducts from rooms of different departments and floors under one deflector. A heat exchanger is provided for heat recovery.

The ventilation system in the ward or unit must be arranged so that a kind of "direct collision" does not arise between the supplied and exhaust air jets, and stagnant zones do not form.

According to the conclusion of the Scientific and Technical Committee of the European Professional Association "Federation of European Heating, Ventilation and Air Conditioning Associations (REHVA)", special attention should be paid to the ventilation of toilets, where a plume containing small drops is formed during flushing. This is why it is important to follow the rule of closing the lid before flushing. In addition, in order to avoid fecal-oral transmission of the pathogen, the exhaust ventilation systems of toilets in the area of the COVID-19 outbreak must operate in a 365/24/7 mode [13].

In the areas where the risk of infection is not so high (resident rooms, nursing staff rooms, auxiliary rooms), a conventional central ventilation system is used, provided that a fundamental rule is applied: a negative pressure gradient must always be left between such areas to completely eliminate the possibility for the contaminated air to pass into the uninfected area. All doors of wards and units must be equipped with a door closer to ensure that the door closing time is minimal. To prevent contaminated air from escaping outside, the end sections of the exhaust ducts should be located as far as possible from the air intake point, as well as places visited by people and animals. In addition, to prevent contaminated air from entering the building, exhaust shafts should be higher than the turbulence zone generated by winds around the building.

It must be taken into account that all filters used in the ventilation system accumulate dust, viruses, bacteria and allergens, and in order to avoid a decrease in the efficiency of air purification, filters must be regularly changed, at least once every 3 months. Disposal of filters is carried out by burning them in special ovens. Also, special attention should be paid to timely maintenance, cleaning and disinfection of ventilation systems, which should be carried out at least once a year, and air intake shafts - at least once every six months. All of the above work must be performed by a specialized organization.

According to SanPiN 2.1.3.2630-10 "Sanitary and Epidemiological Requirements for Organizations Carrying out Medical Activities" [14], in order to reduce air contamination in medical organizations, UV radiation exposure technologies are used. At the same time, the opinion of an epidemiologist, candidate of medical sciences E. I. Sisin on the following disadvantages of using UV technology for air disinfection are as follows [15]:

– The efficiency of irradiation decreases at high humidity, dust, low temperatures of the supplied air;
– Odors and organic pollution are not removed;
– Mercury lamps do not affect mold fungi;
– The use of ozone lamps requires regular ozone measurements;
– The bactericidal flow changes during operation, it must be controlled;
– Increased requirements for the operation and disposal of irradiators that contain mercury;
– High cost of installation and difficult maintenance of flash xenon lamps.

At the same time, the experience of Israeli scientists on the use of UV disinfecting irradiation in the hospital red zone at the Israeli Clinic "Mayanei Hayeshua" during the period of the COVID-19 is of great interest. With a properly set algorithm for the wavelength of UV radiation, the effect is achieved when the operating open UV lamp does not burn the skin, and at the same time, it effectively kills the virus, does not form ozone, which is dangerous for the affected lungs of the patients. Along with this, there was no case of transmission of the COVID-19 from patients to the staff [16].

To ensure smooth operation of the supply and exhaust ventilation and air conditioning systems, in accordance with the requirements of SP 60.13330.2012, clause 7.2.8. [17], and SP 158.13330.2014, section 7.2.3. [18] of health facilities, 50% of the power of the main fans and air conditioning systems are to be backed up.

Access control system (ACS) for premises and zones of inpatient facility for infectious diseases

An inpatient facility for infectious diseases is an object with special requirements for access both to the territory and to the premises. In order to organize gateways separating the air environments of the premises and to implement other requirements of SP 158.13330.2014 "Buildings and rooms for health care facilities. Design rules", it is advisable to use an ACS.

The ACS is a set of technical means aimed to control the entrance to and exit from premises in order to ensure safety and to regulate visits. It allows restricting access to buildings and premises to a certain circle of people (considering their rights to have access). This significantly increases the public safety of the building and, in addition, it can be used to track individual working hours.

In inpatient facility for infectious diseases, in order to ensure the "hands free" principle, it is recommended to use contactless readers for access the premises, which allows the access card to be kept in the breast pocket.

More detailed information on the ACS structure is set out in the article of the Group of Companies "TwinPro" "Isolation and Diagnostic (Individual) Box in the Infectious Department of the Hospital; typical configuration of ACS" [2].

In accordance with the Order of the Government of the Russian Federation dated March 25, 2015 No. 272 amended on April 7, 2020, "Requirements for Anti-Terrorism Protection of Places of Mass Stay of People..." [19], hospitals for infectious diseases are classified as the second class of significance of damage from terrorist threats, as well as the requirements of section 7.6.12; "Television Surveillance Systems" of SP No. 158.13330.2014 [18]. These circumstances require both outdoor video surveillance of the adjacent

territory along the entire perimeter, and in the corridors and entrances to the building and individual units.

For video surveillance it is advisable to use video cameras with high sensitivity and resolution, at least 600 – 800 TVL, with a Day/Night function and infrared illumination. The video surveillance system should allow identification of persons, which will allow timely control of unauthorized leaving of the unit by the patient or presence of unauthorized persons.

Barcoding

In order to optimize the working time of the medical personnel and labor costs, it is advisable to use barcoding technology in medical facilities, by applying graphic information on the medical history, control bracelets for patient identification, labware for analysis, thermal trays, table-feeding, sterilization kits and so on.

Label printers, barcode scanners, data collection terminal, etiquette guns and applicators are used to organize barcoding.

Telemedicine System

In inpatient facilities for infectious diseases in Moscow, the hospital telemedicine system has proven well for constant communication through gadgets between the doctors, between the attending doctor and patients, during a hospital stay, as well as for observation and treatment after the patient is discharged to quarantine (self-isolation).

Operational communication

Experience of the Voronovskoye Moscow Clinical Center for Infectious Diseases, especially in the context of the COVID-19 pandemic, showed an urgent need for operational communication between medical workers, especially in the red zone and the admission office. To ensure operational communication, portable, wearable, waterproof (disinfectable) radio stations forbidden to be carried outside the red zone were used, operating in the 27 MHz radio frequency range (C-B), with an output of 10 W that are not subject to registration with the State Committee for Radio Frequencies and are allowed for free use.

A wireless local area network (Wi-Fi) in inpatient facilities for infectious diseases allows doctors to use tablets to take notes directly in the red zone (paper notes are not allowed in the red zone) and transfer them to a computer located outside of the red zone.

Routing and symbols (Navigation)

Particular attention should be paid to the division and designation of zones in inpatient facilities for infectious diseases. Informing and warning the medical personnel about danger and high risk of infection is highly important in case of COVID-19. Warning and danger signs and posters are used for information purposes. Floor, wall and ceiling navigation systems of various colors are widely used in inpatient facilities for infectious diseases.

Patient nutrition

In terms of quality and safety, a tray feeding system or "tablett feeding" (in German tablett means a tray) in inpatient facilities, based on individual portioning of ready-made meals, not at the department, has proven to be the best under the current circumstances.

The use of the tray feeding system in hospitals for infectious diseases allows organizing the transportation and portioned distribution of food to individual requirements of the patient. Thus, hot dishes are served hot and cold ones cold.

The tray feeding system has the following advantages:

– Significant saving of useful space at the medical department, as well as the whole in-patient facility, since pantries and dishwashing are not required;

– Hygienic safety is ensured when preparing and completing dishes;

– The initial temperature of the dishes is maintained up to 60 minutes due to use of thermal trays with effective thermal insulation;

– The tray feeding system allows using a barcoding system for individual completing of trays at the catering unit, for each patient in accordance with a personal dietary menu, as well as when distributing thermal trays to patients;

– Optimizing of the time for preparing meals for patients (preparing thermal trays for a hospital with a capacity of 200 beds takes about 40 minutes).

Tray feeding is economically feasible and epidemiologically safe due to disposable tableware and cutlery. Disposal of tableware and cutlery is carried out by incineration in special installations (ovens).

Disinfection department and bed decontamination

In hospitals for infectious diseases with a capacity of 100 beds or more, it is practical to arrange a disinfection department for decontamination of adjustable beds in the red zone, which will make it possible not to take the bed out of this zone. Therewith, for passage of the personnel and transportation of disinfectants to the disinfection department, it is necessary to provide an additional entrance to the red zone. The disinfection department and decontamination of adjustable beds should be separated into the clean and the non-clean zones.

In the non-clean zone, the used adjustable beds are received from the red zone departments, disassembled, infected hospital linen (blankets, mattresses and pillows) are transferred for disinfection and bedding is transferred to disinfection and subsequent laundry, so the beds themselves are disinfected.

Before laundry, the infected linen must be disinfected in a room specially designed for disinfection. It is advisable to carry out such disinfection of

linen in a pass-through washing machine, especially used for this purpose.

The clean zone of the disinfection department contains a sterile temporary storage warehouse, as well as service and utility rooms.

For safe mechanized disinfection and decontamination of adjustable beds there should be arranged a walk-through installation for cleaning and disinfection of hospital beds, which is in the interior partition wall in such a way that the loading of dirty beds is made from one side of the installation, and unloading takes place from the front side in the premise of clean beds. The bed streams are completely split.

For the disinfection of infected mattresses, pillows and linen, a passage disinfection chamber should be arranged in the interior partition wall in such a way that the loading of dirty mattresses, pillows and linen is carried out from one side of the installation, and unloading occurs from the front side in the premise of clean beds. Hospital linen streams are completely separated.

The walk-through installation for hospital beds cleaning and disinfection is also used for cleaning and disinfection of hospital carts (if any used).

Upon the impelled transitions from the non-clean zone to the clean one, the operator must pass through the sanitary gate separating the zones.

In the clean zone of the bed disinfection station, completing of clean beds, clean hospital linen and clothes is conducted, as well as temporary storage of clean beds until it is transferred to the department.

For disinfection of individual patient units and other premises of the inpatient facility, compact machines (installations) for aerosol disinfection are recommended.

A special laundry for disinfection and cleaning of ambulances should be arranged on the territory of inpatient facilities for infectious diseases. It is advisable to use the aerosol disinfection. The interior of the car, its equipment and the outer surface are subject to disinfection. It is best to automate this process that will exclude additional time of contact of the personnel with the infected vehicles and it will significantly increase the quality and efficiency of decontamination. For example, using an automatic decontamination system on the entry point and a portable washing system of the BESTWASH CB 720 type, a washing portal or an analogue, with automatic treatment on the entrance, with a capacity of 7 – 8 cars per hour, with a full cycle of cleaning and drying of sanitary vehicles, is carried out.

Medical waste disposal

Medical waste disposal in inpatient facilities for infectious diseases, especially of extremely epidemiologically hazardous class B waste, is the most important factor for public health safety and prevention of epidemic spreading [14]. The machine for disinfection and disposal of class B medical waste on the territory of an inpatient facility for infectious diseases should be away from the main buildings, and the infected waste should not be transported outside its territory.

In this regard, the following modern Russia-made equipment for neutralization of class "B" waste, which is completely harmless and meets international environmental safety standards, is of interest:

– Incinerators for biological and medical waste of classes "B" and "C";

– Equipment for thermal disinfection/neutralization by way of dry hot air [20].

Wastewater treatment facilities

According to the requirements of the SP 30.13330.2016 SNiP 2.04.01-85* Code of Practice, water supply and drainage systems in buildings (approved and put into effect by the Order of the Ministry of Construction of Russia dated 16 December 2016 No. 951/pr) [21], sewage facilities are mandatory requirements for inpatient facilities for infectious diseases. The sewage facilities capacity is calculated with regard to the established standard - 240 liters of wastewater per person. Wastewater is subject to mandatory disinfection.

Considering the experience of the European Union, in inpatient facilities for infectious diseases, thermal disinfection (thermal sterilization) machine for disinfection of wastewater is advised [15].

It is not recommended to use chemical disinfection with chlorine, because a huge amount of chlorine is necessary. Since chlorine reacts with the wastewater content, which leads to formation of a large amount of toxic substances based on chlorine compounds and disrupt the treatment system at the biological treatment plant, an additional dechlorination unit will be needed.

Laundry

The laundry of the inpatient facility for infectious diseases, where sterile cleanliness is required, should be clearly divided into the clean and non-clean zones. Automation throughout the technological laundry process should be of utmost importance. Upon the impelled transitions of the operator from the non-clean zone to the clean, he must pass through the sanitary gate separating the zones. Employees working in the non-clean zone must wear special waterproof overalls, respirators or masks and goggles.

Laundries should be equipped to prevent the risk of spreading infections by accidentally crossing streams of dirty and clean laundry. In this regard, it is necessary to install washing machines of a special type - barrier machines. The use of barrier washing machines is recommended by the methodological guidelines MU 3.5.736.-99, "Technology for processing linen in healthcare facilities" [22].

The barrier washing machine are built in the interior partition wall so the loading of dirty linen is done from one side of the machine, and unloading takes place from the front side in the premise with the clean linen. The streams of linen should be com-

pletely separated and contact of dirty linen with clean linen completely excluded. This reduces the risk of spreading nosocomial infections by 30% to 50% and allows maintaining the required level of cleanliness in the premises.

Hotel-type housing for the medical personnel

Medical personnel working in inpatient facilities for infectious diseases are at high risk of COVID-19 infection and other highly infectious deceases.

In these conditions, it is important to protect persons in contact with medical staff from the risk of infection, to limit contacts of employees of inpatient facilities with the world outside the hospital, and to provide their temporary accommodation in hotel-type housing. Given the above, during construction or recondition of hospitals and departments of infectious diseases, it is recommended to plan a construction of hotel-type housing with sanitary facilities in the rooms (washbasin, shower, toilets) for employees. The number of rooms should equal at least 25 % of the bed capacity.

In order to prevent cross-contamination in hotel-type housing for health personnel, it is necessary to exclude the flow of air from one room to another, and also to provide the possibility of air disinfection and regular disinfection of surfaces in rooms and sanitary facilities.

It is recommended to use disposable tableware and ready meals that are to be delivered in lunch boxes.

Conclusion

The operation of healthcare facilities providing medical care to infectious patients during the pandemic of the coronavirus disease 2019 revealed the shortcomings of the current regulatory documents (Construction Rules and Regulations, Sanitary Rules and Regulations, Code of Practice, etc.), and introduced new requirements for the organization of work in such facilities.

Prevention of spread of infectious diseases and nosocomial infections among medical personnel and patients necessitates improvement of the regulatory framework of the medical and technical aspects of inpatient facilities for infectious diseases.

References

1. Gotie SV, Revishvili ASh, Pushkar DY, Adamyan LV, Krylov VV, Shelygin YA, et al. Emergency surgery in the circumstances of COVID-19 [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://xn----7sbgcd3afnu7aa9ax5f.xn--p1ai/docs/metodrec/covid-19-recommendation.pdf.

2. Isolation and diagnostic (Meltzer) box in the infectious diseases department of the hospital. Typical ACS configuration [Internet]. 2020 [cited 2020 Jun 5]. Available from: http://www. trevog.net/news/2651/.

3. Приказ Министерства здравоохранения и социального развития РФ от 31 января 2012 г. N 69н «Об утверждении Порядка оказания медицинской помощи взрослым больным при инфекционных заболеваниях» (с изменениями и дополнениями) (Order of the Ministry of Health Care and Social Development of the Russian Federation dated 31 January 2012 No. 69n "On Approving the Procedure for Rendering Medical Assistance to Adult Patients Having Infectious Diseases") [Internet]. 2012 [updated 2020 Feb 21; cited 2020 Jun 5]. Available from: http://base.garant.ru/70158576/#friends.

4. Akhiyarov I. New era of medical assistance [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://yandex.ru/turbo/s/resbash.ru/articles/cotsium/Novaya-era-meditsinskoypomoshchi-298370/.

5. Scientists from Sarov have created an apparatus for treating patients with coronavirus by nitric oxide [Internet]. 2020 [cited 2020 Jun 2]. Available from: https://sdelanounas.ru/blogs/132574/.

6. "Tianox" against coronavirus [Internet]. 2020 Apr 28 [cited 2020 Apr 28]. Available from: http://sarov.ru/articles/view/tianoks-protiw-koronawirusa.

7. Bateneva T. Академик Александр Чучалин рассказал "РГ" о тайнах коронавируса (Academic Alexander Chuchali has told the «Russian Newspaper» about the secrets of coronavirus) [Internet]. 2020 [cited 2020 Jun 2]. Available from: https://rg.ru/2020/04/19/nauka-shag-za-shagom-otkryvaet-tajny-koronavirusa.html.

8. Пациентов с коронавирусом в Москве лечат с помощью барокамер (Altitude chambers are used in Moscow to treat patients with COVID-19) [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://tass.ru/moskva/8654443.

9. Derjagin P. Therapy in an altitude chamber is one of the ways to help seriously ill patients with coronavirus [Internet - video]. 2020 [cited 2020 Jun 5]. Available from: https://www.1tv.ru/news/2020-04-25/384646-terapiya_v_barokamere_odin_iz_sposobov_pomoch_tyazhelym_patsientam_s_koronavirusom.

10. В Москве для лечения пациентов с COVID-19 используют барокамеры [Altitude chambers are used in Moscow to treat patients with COVID-19] [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://www.mos.ru/news/item/75152073/.

11. ОМУ 42-21-27-88 Аппараты гипербарической оксигенации. Правила эксплуатации и ремонта (Hyperbaric oxygenation therapy apparatus. Rules for use and repair) [Internet]. 1989 [cited 2020 Jun 5]. Available from: http://docs.cntd.ru/document/1200067932.

12. Vergani C. Системы воздухоподготовки в инфекционных отделениях больниц (Air conditioning systems in hospital infectious diseases) [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://www.abok.ru/for_spec/articles.php?nid=2472

13. REHVA COVID-19 guidance document, April 3, 2020:how to operate and use building services in order to prevent the spread of the coronavirus disease (COVID-19) virus (SARSCoV-2) in workplaces [Internet]. 2020 [cited 2020 Jun 2]. Available from: https://www.rehva.eu/fileadmin/user_upload/REHVA_COVID-19_guidance_document_ver2_20200403_1.pdf

14. СанПиН 2.1.3.2630-10 Санитарно-эпидемиологические требования к организациям, осуществляющим медицинскую деятельность (On the approval of SanPiN 2.1.3.2630-10 Sanitary and epidemiological requirements for organizations carrying out medical activities) [Internet]. 2010 [cited 2020 Jun 5]. Available from: http://docs.cntd.ru/document/902217205.

15. Sisin EI. Comparing air disinfection technologies in medical organizations. Sanepidcontrol. Labor protection [serial on the Internet]. 2016 [cited 2020 Jun 2];(2). Available from: https://www.profiz.ru/sec/2_2016/tehnologii_obezzarazh/.

16. Ultraviolet disinfection system is being introduced in Israel [Internet]. 2020 [cited 2020 Jun 2]. Available from: https://ria.ru/20200602/1572327156.html.

17. SP 60.13330.2016 Heating, ventilation and air conditioning. Updated edition of SNiP 41-01-2003 (with Amendment No.1) [Internet]. 2017 [cited 2020 Jun 5]. Available from: http://docs.cntd.ru/document/45605420.

18. SP 158.13330.2014 Buildings and premises for health care facilities. Design rules. With Amendment No. 1. [Internet]. [cited 2020 Jun 5]. Available from: https://gostperevod.com/sp158-13330-2014.html.

19. Resolution of the Government of the Russian Federation of 25.03.2015 N 272 "On approval of the requirements for anti-terrorist protection of places of mass stay of people and objects (territories) subject to mandatory protection by the troops of the National Guard of the Russian Federation, and forms of security passports of such places and objects (territories) "/ / "Collection of Legislation of the Russian Federation", 06.04.2015, N 14, Article 2119. [Internet]. https://base.garant.ru/70937940/

20. Medical waste: classification and treatment rules [Internet]. 2020 [cited 2020 Jun 5]. Available from: https://medservise24.ru/blog/medotkhody-i-obrashchenie-s-otkhodami/ meditsinskie-otkhody-klassifikatsiya-i-pravila-obrashcheniya/.

21. СП 30.13330.2016 Внутренний водопровод и канализация зданий. Актуализированная редакция СНиП 2.04.01-85* (с Поправкой, с Изменением N 1) (On approval of SP 30.13330 "SNiP 2.04.01-85*. Domestic water supply and drainage systems in buildings) [Internet]. 2017 [cited 2020 Jun 5]. Available from: http://docs.cntd.ru/document/456054201.

22. «MU 3.5.736-99. 3.5. Disinfectology. Technology for processing linen in medical institutions. Methodological instructions» (approved by the Chief State Sanitary Doctor of the Russian Federation 03.16.1999) [Internet]. [cited 2020 Jun 5]. Available from: http://www.consultant.ru/document/cons_doc_LAW_130246/.

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Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

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.

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

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– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

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