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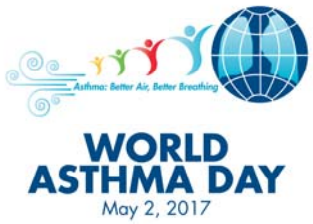

World Asthma Day has been marked on the international level since the year 1998 after the first "World Asthma Meeting" in Barcelona (Spain) every first Tuesday in May. This event is organized by the Global Initiative for Asthma (GINA) with purpose to increase asthma awareness and improve its care around the world. World Asthma Day focuses on educating the public on the most effective ways of controlling and treating asthma (see Editorial, pp. 399–401).

Svetski dan astme obeležava se na internacionalnom nivou od 1998. godine, posle prvog Svetskog mitinga o astmi održanog u Barseloni (Španija), svakog prvog utorka u mesecu maju. Ovaj događaj organizuje Globalna incijativa za astmu sa ciljem (GINA) da se širom sveta poveća svest o astmi i poboljša lečenje obolelih od nje. Svetski dan astme ima u fokusu upoznavanje javnosti sa najefikasnijim načinima kontrole i lečenja ove bolesti (vidi Uvodnik, str. 399–401).

E D I T O R I A L

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Bronchial asthma – from psychosomatic illness to proinflammatory cytokines and asthma phenotypes

Bronhijalna astma – od psihosomatske bolesti do proinflamatornih citokina i fenotipova astme

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Bronchial asthma is a chronic inflammatory heterogeneous disease of the airways that is clinically manifested in episodes of heavy breathing, especially at the phase of expirium, shortness of breath accompanied by wheezing, and cough and expectoration of a thick, tough and sticky secretions [1]. Pathogenically, asthma is characterized by chronic inflammation of bronchial mucosa and bronchial hypersensitivity, which result in the appearance of the variable, predominantly obstructive disorder of pulmonary ventilation, or by reducing the air flow rate while breathing [2–4]. Asthma was known in ancient times, in Egypt and in ancient Greece, as evidenced by the writings of Hippocrates (460–370 BC; άσθμα - gasping). It is present in 1–18% of the population, depending on the countries and regions [1]. During 2004, it was estimated that asthma affects about 300 million people worldwide of all age structure, and it is estimated that by 2025 some 100 million people more will have this disease [5, 6].

Knowledge of the etiology and pathophysiology of events in asthma has evolved with the progress of medical science: from data of "hay fever" from the 19th century through the theories of "psychosomatic disease" from the first half of the 20th century [7, 8] to the present knowledge of inflammation and bronchial hypersensitivity and hyperreactivity.

Risk factors for the onset, development and exacerbations of asthma are divided into host factors and environmental factors. Host factors are predisposing factors for the development of inflammation, which in conjunction with other factors can lead to the clinical manifestations of the disease. They are: genetic predisposition, atopic constitution, airway hyperreactivity, gender and race/ethnic factor. Environmental factors are usually responsible for the manifestation of the disease: allergens indoors and outdoors as well as in the workplace, smoking, air pollution, respiratory infections, parasitic infections, socio-economic status, drugs, food additives and obesity.

Bronchoconstriction is responsible for both bronchospasm and increased mucosal secretion and mucosal edema of the airways for the occurrence of symptoms of asthma [1]. The main underlying pathophysiological mechanism of asthma is the airway inflammation, and occasionally variable airway obstruction and bronchial hyperreactivity are its feature manifestations. Inflammation of the airways includes interaction of a large number of mechanisms: hyperresponsiveness, mucosal edema of the respiratory tract, bronchial gland hypersecretion and increased production of mucus, hypertrophy of the smooth muscle cells and airway remodeling which represents an irreversible process due to collagen deposition.

In the development and maintenance of chronic inflammation in the airways following factors are participating: inflammatory and structural cells, neuroregulatory substances and mediators (histamine and chemotactic factors, leukotrienes and many cytokines [granulocyte macrophage stimulating factor, tumor necrosis factor, interleukins (IL) - 1, 2, 3 , 4, 5, 6, 8, 11, 13, 17 and others] [9–17].

For the inflammation in asthma, the most important cells are activated mast cells, activated T-lymphocytes as regulatory cells and eosinophils. In the early asthmatic reaction, in sensitized persons, upon exposure to the allergen and its binding to specific antibodies, activated mast cells secrete mediators of the acute phase (which include leukotrienes and inflammatory cytokines) which have a role to maintain the inflammatory phase [17]. They also release IL-5, leading to a Th2 lymphocyte differentiation, chemotaxis and differentiation of eosinophil leukocytes, modification of basophil activity, and activation of the enzyme tryptase, which results in the further development of the inflammation in the airways [14, 15] . Mobilized and activated eosinophils play a central effector role in the development and

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maintenance of inflammation in asthma by producing a number of mediators, including the major basic protein, eosinophil cationic protein (ECP), eosinophilic neurotoxin and eosinophil peroxidase [12, 13]. A ribonuclease and ECP, act on the respiratory epithelium and pneumocytes leading to damage of the epithelial barrier and activation of adhesion molecules, mobilization of other inflammatory cells, and stimulation of fibroblasts [16–19].

The diagnosis of asthma is based on the clinical examination (complete physical examination, medical history of difficulty in breathing, and a complete history of the disease, with a very significant abnormal physical findings in the lungs), lung function tests (spirometry, the peak expiratory flow, bronchodilatory and bronchial provocation tests - specific or non-specific) that can confirm the existence of asthma and the existence of the variable flow restriction of air through the airways and allergy tests (determination of IgE specific antibodies and skin prick tests) which can identify the atopic constitution and a factor that is the cause of the attack of asthma. In some patients, radiologic diagnostics is used, which is important as a method which can exclude the existence of any other pulmonary disease. In the diagnosis of asthma, for research purposes, and today in many centers during routine analysis, the measuring of certain inflammation markers is used (in a sample of sputum or induced sputum, nasal swabs, biopsy specimens of the bronchus or after the bronchoalveolar lavage inflammatory cells and cytokines are determined as well as measurement of the mediators and their metabolites in blood and urine and measuring the concentration of nitric oxide (NO) in the exhaled air] [1, 16, 20–24.] Variability of disorders of the pulmonary ventilation is determined by the spontaneous evolution, bronchodilatatory tests and proving of hypersensitivity [1, 25, 26].

For testing of pulmonary function in patients with asthma, spirometry is the most often used test to determine the values of the parameters of lung ventilation: forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and the ratio of FEV1/FVC. In the manifested asthma, we most often register the obstructive ventilatory impairment (decrease in FEV1) and in such cases a bronchodilatatory test is routinely performed in order to verify variability of the disorders. In the diagnosis and monitoring of asthma other methods may be used such as flow-volume curve, peak expiratory flow measurement peak expiratory flow – (PEF) , body plethysmography, lung compliance, determining the value of the transfer factors of the lungs, arterial blood gas analysis with acid-base status and pulse oscillometry [1, 24–28].

Asthma is a chronic, incurable disease based on a continuing inflammation in the airways, which varies in intensity and clinical manifestations, either spontaneously or as a result of the applied treatment. Therefore, clinicians are recommended the classification of asthma by the level of disease control in mild, moderate, severe and very severe, depending on the value of FEV1 and FEV1/FVC [29].

For the scientific and research purposes asthma is classified according to the frequency of symptoms into intermittent and persistent. In intermittent asthma patients have occasional complaints, spirometry test out of episodes of attacks shows no disorders and bronchial provocation tests are usually negative [1, 30]. Problems occur less than once a week, exacerbations are short lasting, and the values of PEF and FEV1 are greater than 80% of the predicted ones with the variability of less than 20%. Persistent asthma can be mild, moderate, and severe. Mild persistent asthma is characterized by the onset of symptoms more than once a week, night attacks more than twice a month, and the values of PEF and FEV1 greater than 80%, with a variation of 20–30%. In severe stages the symptoms are constantly present, there are frequent exacerbations and nocturnal seizures, limited physical activity, and the PEF and FEV1 values less than or equal to 60%, while the variability is greater than 30%.

Based on the clinical and functional characteristics, inflammatory-immune profiles and capabilities for the treatment and control of diseases with medicaments and other methods, the population of patients with asthma is divided into the following phenotypes: allergic bronchial asthma with early onset and dominant eosinophilic inflammation – responds well to anti-inflammatory therapy of glucocorticoids; non-allergic asthma – characterized by inflammation with of neutrophil and eosinophil leukocytes involvement; late-onset asthma - needs high doses of inhaled glucocorticoids for the control; asthma with fixed airflow limitation; asthma associated with obesity [29].

Treatment of asthma is focused on relief of symptoms and alleviating chronic inflammation and bronchial hyperreactivity. Combining multiple pharmacological substances, either in terms of anti-inflammatory drugs, bronchodilators or specific drugs that act on particular inflammation mediators provides better control of symptoms and improves the quality of life. At the same time, it reduces the risk of severe asthma attacks with suffocation and a sense of lack of air as well as the chance of a fatal outcome. The therapy starts when the disease is confirmed by pulmonary function tests along with a history of typical symptoms.

For clinical practice, asthma will continue to be a challenge in patients with a history of chronic symptoms and who have a normal pulmonary ventilation determined by the spirometric test and negative tests to bronchial hyperreactivity because some of them already have intermittent asthma (which due to the spontaneous variability of symptoms, inflammation and hypersensitivity remains undiagnosed and untreated) [31, 32].

REFERENCES

1. Global initative for asthma. 2016 GINA Report, Global Strategy for Asthma Management and Prevention (2016 Update). Available from: http://ginasthma.org/2016-gina-report-global-strategy-for-asthma-management-and- prevention/
2. *Fish JE, Shaver JR, Peters SP*. Airway hyperresponsiveness in asthma. Is it unique? Chest 1995; 107(3 Suppl): 154S–6S.
3. *O'Byrne PM, Inman MD*. Airway hyperresponsiveness. Chest 2003; 123(3 Suppl): 411S–6S.

4. *Borak J, Lefkowitz RY*. Bronchial hyperresponsiveness. Occup Med (Lond) 2016; 66(2): 95–105.
5. *Masoli M, Fabian D, Holt S, Beasley R*. Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004; 59(5): 469–78.
6. *Braman SS*. The global burden of asthma. Chest 2006; 130(1 Suppl): 4S–12S.
7. *Bosfort FH*. Hay fever, asthma, and allied affections. Trans Am Climatol Assoc Meet 1885; 2: 151–70.
8. *Opolski M, Wilson I*. Asthma and depression, pragmatic overview of the literature and guidelines for the future research. Clin Pract Epidemol Ment Health 2005; 1: 18.
9. *Gauvreau GM*, *Watson RM*, *O'Byrne PM*. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. Am J Respir Crit Care Med 1999; 160(2): 640–7.
10. *Pignatti P, Perfetti L, Galdi E, Pozzi V, Bossi A, Biale C,* et al. Increased CD69 expression on peripheral blood eosinophils after specific inhalation challenge. Allergy 2002; 57(5): 411–6.
11. *Djukanović R, Feather I, Gratziou C, Walls A, Peroni D, Bradding P,* et al. Effect of natural allergen exposure during the grass pollen season on airways inflammatory cells and asthma symptoms. Thorax 1996; 51(6): 575–81.
12. *Jeffery PK*. Bronchial biopsies and airway inflammation. Eur Respir J 1996; 9(8): 1583–7.
13. *Corrigan CJ, Kay AB*. T-cell/eosinophil interactions in the induction of asthma. Eur Respir J Suppl 1996; 22: 72s–8s.
14. *Corren J*. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. Discov Med 2012; 13(71): 305–12.
15. *Rosenwasser LJ*, *Rothenberg ME*. IL-5 pathway inhibition in the treatment of asthma and Churg-Strauss syndrome. J Allergy Clin Immunol 2010; 125(6): 1245–6.
16. *Holgate ST*, *Polosa R*. The mechanisms, diagnosis, and management of severe asthma in adults. Lancet 2006; 368(9537): 780–93.
17. *Barnes PJ*. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008; 8(3): 183–92.
18. *Walsh ER*, *August A*. Eosinophils and allergic airway disease: there is more to the story. Trends Immunol 2010; 31(1): 39–44.
19. *Bystrom J, Amin K, Bishop-Bailey D*. Analysing the eosinophil cationic protein--a clue to the function of the eosinophil granulocyte. Respir Res 2011; 12(1): 10.
20. *Perić A, Vojvodić D, Radulović V, Vukomanović-Đurđević B, Miljanović O*. Correlation between cytokine levels in nasal fluid and eosinophil counts in nasal polyp tissue in asthmatic and non-asthmatic patients. Allergol Immunopathol (Madr) 2011; 39(3): 133–9.
21. *Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemière C, Pizzichini E,* et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J 2006; 27(3): 483–94
22. *Prasad A, Langford B, Stradling JR, Ho LP*. Exhaled nitric oxide as a screening tool for asthma in school children. Respir Med 2006; 100(1): 167–73.
23. *Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC*. Exhaled nitric oxide measurements: clinical application and interpretation. Thorax 2006; 61(9): 817–27.
24. *Schafroth Török S, Leuppi JD*. Bronchial hyper-responsiveness and exhaled nitric oxide in chronic obstructive pulmonary disease. Swiss Med Wkly 2007; 137(27–28): 385–91.
25. *Stangl B*. Nonspecific and specific bronchial provocation tests. Plućne bolesti 1984; 36(1–2): 7–15. (Croatian)
26. *Ninković M, Radojičić M*. Standardization of bronchial provocation tests using histamine. Vojnosanit Pregl 1988; 45(5): 350–2. (Serbian)
27. *Komarow HD, Skinner J, Young M, Gaskins D, Nelson C, Gergen PJ,* et al. A study of the use of impulse oscillometry in the evaluation of children with asthma: analysis of lung parameters, order effect, and utility compared with spirometry. Pediatr Pulmonol 2012; 47(1): 18–26.
28. *Shi Y, Aledia AS, Tatavoosian AV, Vijayalakshmi S, Galant SP, George SC*. Relating small airways to asthma control by using impulse oscillometry in children. J Allergy Clin Immunol 2012; 129(3): 671–8.
29. *Wenzel SE*. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012; 18(5): 716–25.
30. *Cockcroft DW, Murdock KY*. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. Thorax 1987; 42(4): 302–8.
31. *Aćimović LS, Plavec G, Tomić I, Karličić V, Aćimović MS, Vuković J,* et al. Symptoms, Physical findings and bronchial hypersensitivity in patients with bronchial asthma and normal spirometry. Vojnosanit Pregl 2009; 66(1): 39–43.
32. *Aćimović S*. Assessment of the connestion of the clinical status, values of the parameters of the mechanics of respiration, variability in pulmonary ventilation, inflammation and hypersensitivity in intermittent asthma [dissertation]. Belgrade: University of Defense, Military Medical Academy, Faculty of Medicine of the Military Medical Academy; 2016. (Serbian)

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ORIGINAL ARTICLES

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Oral health-related quality of life of institutionalized elderly in Serbia

Kvalitet života povezan sa oralnim zdravljem korisnika domova za stara lica u Srbiji

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***Department of Prosthodontics, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia; †Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Elderly residents in nursing homes have a great risk of periodontal and tooth diseases. Improving oral health can also improve residents' general health and quality of life. The objective of our study was to investigate oral health related quality of life of institutionalized elderly in Serbia using Geriatric Oral Health Assessment Index (GOHAI). **Methods.** The Serbian version of the GOHAI questionnaire was developed in accordance with the recommended backward-forward method. A total of 301 participants completed the Serbian version of the GOHAI questionnaire. The questionnaire sought information about sociodemographic characteristics and self-reported perception of general and oral health. Clinical examination included assessment of periodontal and dental status. Reliability, internal consistency, and concurrent and convergent validity of GOHAI scores were examined. **Results.** There were 197 female and 104 male participants with the average age of 78.6 (SD ± 7.8) and average time spent in nursing home 4.9 (SD ± 4.7) years. The average score of the GOHAI was 48.4 (SD ± 8.4). Low GOHAI scores were associated with perceptions of poor oral and general health. Cronbach's alpha coefficient for the Serbian version of GOHAI was 0.79. This instrument showed a high level of internal consistency and homogeneity between questions. The respondents who perceived that they needed dental treatment at the time had significantly lower GOHAI scores. A total of 48.5% of the participants reported 'always' having difficulties when chewing. On the other hand, a small number of participants (0.3%) used medications 'always' to relieve dental pain. **Conclusion.** The Serbian version of the GOHAI showed acceptable reliability and validity. The GOHAI final score was considered low, indicating low oral health self-perception by the institutionalized elderly in Serbia.

Key words:
periodontics; tooth; aged; homes for the aged; surveys and questionnaires; sensitivity and specificity; serbia.

Apstrakt

Uvod/Cilj. Stariji korisnici domova za stara lica izloženi su velikom riziku od nastanka periodontalnih bolesti i bolesti zuba. Poboljšanje oralnog zdravlja može poboljšati opšte zdravlje i kvalitet života korisnika domova za stara lica. Cilj našeg istraživanja bio je da se ispita oralno zdravlje i njegova povezanost sa kvalitetom života korisnika domova za stara lica u Srbiji pomoću indeksa *Geriatric Oral Health Assessment* (GOHAI). **Metode.** Srpska verzija upitnika GOHAI razvijena je u skladu s preporučenom *backward-forward* meto-dom. Ukupno 301 ispitanik učestvovao je u studiji. Upitnik je sadržao pitanja o sociodemografskim karakteristikama i sopstvenoj percepciji oralnog i opšteg zdravlja. Klinički pregled uključivao je procenu periodontalnog i dentalnog statusa. Ispitivani su pouzdanost, interna konzistentnost i konkurentna i konvergentna valjanost upitnika GOHAI. **Rezultati.** Ispitano je 197 ženskih i 104 muška ispitanika, prosečne starosti od 78,6 (SD ± 7,8) godina sa prosečnim vremenom provedenim u domu od 4,9 (SD ± 4,7) godina. Prosečna vrednost skora GOHAI bila je 48,4 (SD ± 8,4). Nizak GOHAI bio je povezan s percepcijama lošeg oralnog i opšteg zdravlja. Koeficijent Cronbach alfa za srpsku verzi-ju GOHAI iznosio je 0,79. Ovaj instrument je pokazao visok nivo interne konzistentnosti i homogenosti između pitanja. Ispitanici koji su imali potrebu za stomatološkom intervencijom pokazali su značajno niže GOHAI rezultate. Ukupno 48,5% ispitanika imalo je "uvek" poteškoća u toku žvakanja. S druge strane, mali broj ispitanika (0,3%) "uvek" koristi lekove za ublažavanje zubobolje. **Zaključak.** Srpska verzija GOHAI pokazala je prihvatljivu pouzdanost i validnost. Konačni GOHAI rezultat je nizak, što ukazuje na loše oralno zdravlje i sa njim povezan kvalitet života korisnika domova za stara lica u Srbiji.

Ključne reči:
periodontologija; zubi; stare osobe; staračke domovi; ankete i upitnici; osetljivost i specifičnost; srbija.

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Introduction

Despite its relatively recent emergence over the past few decades, oral health-related quality of life (OHRQoL) has important implications for the clinical practice of dentistry and dental research [1]. OHRQoL is a multidimensional construct that includes a subjective evaluation of the individual's oral health, functional well-being, emotional well-being, expectations and satisfaction with care, and sense of self. It is an integral part of general health and well-being and it is recognized by the World Health Organization (WHO) as an important segment of the Global Oral Health Program (2003) [1]. A growing number of elderly people spend the last years of their lives in long-term care facilities [2]. Within the elderly population, there is a greater risk of caries, periodontal disease and teeth loss, especially among functionally dependent and cognitively impaired residents in nursing homes [3]. Therefore, improving oral health can also improve residents' general health and quality of life.

In the past three decades, different OHRQoL have been introduced into clinical practice because it has been shown that the exclusive use of clinical evaluation does not take into consideration the functional and psychosocial aspects of oral health [4]. The most commonly used indexes are Oral Health Impact Profile (OHIP) [5], Oral Impacts on Daily Performance (OIDP) [6] and Geriatric/General Oral Health Assessment Index (GOHAI) [7]. Geriatric Oral Health Assessment Index (GOHAI) was originally developed to assess the oral health of elderly patients. The assessment index consists of a questionnaire composed of 12 questions intended to evaluate following three different aspects of oral health related to quality of life: physical functioning including nutrition, speech, ingestion; psychosocial functioning including oral health care or consideration for oral health, appearance dissatisfaction, self-awareness, avoidance of social contacts due to oral problems, and pain or discomfort including the use of pain relievers [7]. In some studies [8], the GOHAI has been used for self-ratings of dental appearance in the elderly population as well as to assess correlation between the impact of oral diseases to everyday functioning and welfare in two populations of elderly people [9], and it proved to be a sensitive instrument for evaluating the dental treatment success, as well [10].

The objective of our study was to investigate OHRQoL of institutionalized elderly in Serbia using GOHAI index.

Methods

Sample

The research was conducted in the Belgrade Gerontology Center, which consists of 4 nursing homes located in the urban area. Participants were contacted on working days, during January, February and March of 2014. The protocol for this study was approved by the local Ethics committee (No. 36/31) at the Faculty of Dental Medicine University of Belgrade. The inclusion criteria were as follows: the participant should be over the age of 65, a resident of the Belgrade Gerontology Center, and should not have had previously verified cognitive and psychiatric disorders (confirmed by insight into medical documents of the residents). All the patients signed informed consent forms that had been approved by the Ethics Committee. Sample size calculation was based on test-retest reliability measured by the intraclass correlation (r). The assumed expected GOHAI r was 0.7. An r of 0.6 or higher would have been acceptable (Ho : Po = 0.6 and H1 : P1 = 0.7). Using a two-sided test suggested by Walter et al. [11] with $\beta = 0.2$ (80% power) and $\alpha = 0.05$, a sample size of 205 subjects was required. The sample size was increased to 301, assuming a possible dropout rate of 30%.

Serbian version of Geriatric Oral Health Assessment Index

The first step in testing the OHRQoL using the GOHAI was to create a Serbian version of the questionnaire. It was developed in accordance with the recommended internationally used method, which consists of a cross-cultural adaptation process [12]. The process is comprised of forward translation, back translation, pre-testing and a final version [12]. The draft of the Serbian version of GOHAI was obtained by translation of the English version by four dentists who were involved in the study. Subsequently, a consensus version in Serbian was obtained. The consensus Serbian version was translated into English by a professional translator who was not involved in the study but was familiar with the dental terms. A back translation was performed without previous knowledge about the original text in English. The two obtained versions of GOHAI (Serbian and English) were reviewed by the entire research team and a professional translator. After reconciliation of opinions, a preliminary Serbian version of the GOHAI questionnaire was obtained. The next step in the verification of this index was a pilot survey, which implied examination of intelligibility of the questions by the elderly population. The sample for the pilot survey consisted of 20 respondents of older age who were patients at the Clinic for Prosthetic Dentistry at the Faculty of Dental Medicine in Belgrade. The pilot study showed that the questionnaire had been carefully designed and that the questions had been precisely translated. After that, a final Serbian version of the GOHAI questionnaire was obtained. Internal consistency and homogeneity of the translated GOHAI was assessed based on Cronbach's alpha. To test the concurrent validity of the translated GOHAI, the answers to self-perceived questions related to the self-assessment of general health, oral health and need for dental treatment were used. It was assumed that people with different answers to these questions would have different GOHAI scores.

Oral health related quality of life assessment

The participants were asked nine negatively worded and three positively worded questions. There were five categories of answers for each question and a score was assigned to each category (1 = always, 2 = often, 3 = sometimes, 4 = rarely and 5 = never). The results from positively worded questions were reversed during data processing, so that the directions of all answers were the same. The GOHAI score

was calculated by adding the results of all the answers for 12 questions. Therefore, the GOHAI score ranged from 12 to 60 with higher scores indicating better oral health.

In addition to the 12 questions within the GOHAI, the questionnaire consisted of questions related to personal data and self-perceived questions of oral and general health. These self-perceived questions were necessary for the psychometric analysis of the questionnaire (oral hygiene of the participants, self-perception of their oral and general health, the need for dental treatment, the presence of toothache, gum bleeding, bad breath, dry mouth, pain in the temporomandibular joint and chewing disability).

Then, the participants were clinically examined in accordance with procedures and criteria for diagnosis as recommended by the World Health Organization [13]. Clinical examination included the number of decayed and missing teeth.

Statistical analysis

The data were analyzed using statistic software SPSS, version 11.5 for Windows, SPSS Inc. Chicago, IL, USA. Cronbach's alpha was calculated to assess the degree of internal consistency and homogeneity between the items. The intraclass correlation coefficient (ICC) was used for the assessment of test-retest reliability. Pearson's correlation coefficient was used for examination of the correlation between the self-perceived general and oral health status and the need for dental treatment with the total GOHAI score, which was the rating of concurrent validity. Additionally, the Kruskal-Wallis test, the Mann-Whitney U-test and the *t*-test were used for the convergent validity. Statistical significance was determined as $p < 0.05$.

Table 1
Sociodemographic and other important characteristics of the participants (n = 301)

Variable	n	%
Gender		
male	104	34.6
female	197	65.4
Mean age (years)	78.59	
Educational level		
less than high school	98	32.6
high school or more	203	67.4
Mean time spent in nursing home (years)	4.96	
Functionally dependent elderly		
yes	111	36.8
no	190	63.1
if answer is yes, how long? (mean in years)	4.82	
Maintenance of oral hygiene		
independently	244	81.1
with the assistance	7	2.3
does not maintain	50	16.6
Tooth brushing		
< twice daily	159	52.8
≥ twice daily	142	47.2
The use of additional funds		
yes	12	4.0
no	289	96.0
Frequency of dental visits		
< once a year	274	91.0
≥ once a year	27	9.0
Last visit to the dentist		
within last year	65	21.6
1–3 years	45	15.0
3–5 years	31	10.3
5–10 years	79	26.2
> 10 years	81	26.9

Results

Characteristics of the participants

A total of 301 people were clinically examined and interviewed, and their data included into the analysis. There were 197 female and 104 male participants and all of them were between 65 and 100 years of age, with the average age of 78.6 (SD ± 7.8) years. The average time spent as a resident of a home was 4.9 (SD ± 4.7) years. Other sociodemographic characteristics are presented in Table 1.

Reliability

The Cronbach's alpha coefficient for the Serbian version of the GOHAI was 0.79. Inter-item correlation coefficients between GOHAI questions ranged from 0.04 to 0.67, while the mean value of inter-item correlation was 0.24 (Table 2).

The test-retest correlation coefficient ranged from 0.40 to 0.85 for each individual question. The test-retest correlation coefficient for the total GOHAI result was 0.66, which indicated good stability.

Validity

Concurrent validity for the GOHAI was evaluated by determining the correlation between the self-perceived general and oral health status and the need for dental treatment with the total GOHAI score (Table 3). Worse general and oral health perception resulted in lower GOHAI scores. Moreover, the respondents who perceived that they needed dental treatment at the time had significantly lower GOHAI values, which indicated that bad oral health was related to quality of life.

Lower GOHAI results were related to self-reported toothache, sensitivity to hot and cold, dry mouth, bad breath and inability to chew food, which supports the convergent validity (Table 4). The participants with one or more missing or decayed teeth had lower GOHAI scores than those with no missing or decayed teeth.

Self-perception of oral and general health

The majority of the participants (44.2%) thought that their general health was good, while 7% rated their health as very good. Speaking of oral health, 60.5% thought it was good, while 5% rated it as very good. Overall, 44.9% of the participants considered that they needed a dental treatment, and according to the participants' perception the most common oral problems were dry mouth (59.5%) and sensitivity of teeth to hot and cold (20.6%). Self-assessment of general and oral health is presented in Table 5.

Clinical examination showed that 27.2% of the examinees had 1 or more decayed teeth. The average number of missing teeth was 25.2 (SD ± 8.2), while 99.7% of the examinees had 1 or more missing teeth.

Table 2

Reliability analysis of Geriatric Oral Health Assessment Index: corrected item-total correlation, Cronbach's alpha and alpha if item deleted (n = 301)

Item	Corrected item-total correlation	Alpha if item deleted	Test-retest correlation*
Limit foods	0.49	0.76	0.85
Trouble biting, chewing	0.39	0.78	0.78
Swallow comfortably	0.29	0.78	0.49
Trouble speaking	0.44	0.77	0.77
Eat without discomfort	0.53	0.76	0.61
Limited social contacts	0.51	0.77	0.84
Pleased with appearance	0.42	0.77	0.83
Use of medication	0.30	0.78	0.40
Worry/concern	0.50	0.76	0.78
Nervous/self-conscious	0.51	0.76	0.58
Uncomfortable eating with people	0.56	0.76	0.49
Teeth or gums sensitive	0.24	0.79	0.50
$\alpha = 0.79$			

***Intraclass correlation coefficient.**

Table 3

Concurrent validity: A correlation between self-reported general and oral health and the need for dental care and the Geriatric Oral Health Assessment Index (GOHAI) scores (n = 301)

Item	GOHAI score (mean ± SD)	Pearson's correlation coefficient
Self-reported general health		
very bad (n = 25)	41.0 ± 8.4	
bad (n = 122)	47.8 ± 8.2	
good (n = 133)	49.9 ± 8.3	0.27
very good (n = 21)	51.4 ± 5.1	
Self-reported oral health		
very bad (n = 15)	41.8 ± 8.2	
bad (n = 89)	44.4 ± 8.2	0.36
good (n = 182)	50.7 ± 7.6	
very good (n = 15)	50.8 ± 8.5	
Self-reported need for dental treatment		
yes (n = 135)	45.2 ± 8.9	
no (n = 166)	51.0 ± 7.1	0.35

Table 4

Convergent validity: differences in the average of the Geriatric Oral Health Assessment Index (GOHAI) scores according to self-reported responses to different health-related questions and objective assessment of oral health (n = 301)

Item	GOHAI score (mean ± SD)	*p*-value
Self-reported toothache		
yes (n = 43)	42.8 ± 7.5	$< 0.05^a$
no (n = 258)	49.3 ± 8.2	
Self-reported sensitivity to hot and cold		
yes (n = 62)	43.4 ± 8.6	$< 0.05^a$
no (n = 239)	49.7 ± 7.9	
Self-reported TMJ pain		
yes (n = 20)	44.9 ± 8.5	0.52^b
no (n = 281)	48.6 ± 8.4	
Self-reported bleeding gums during brushing		
yes (n = 34)	46.1 ± 9.4	0.15^b
no (n = 267)	48.7 ± 8.3	
Self-reported dry mouth		
yes (n = 179)	46.9 ± 8.5	$< 0.05^b$
no (n = 122)	50.6 ± 7.9	
Self-reported bad breath		
yes (n = 53)	46.1 ± 9.3	$< 0.05^b$
no (n = 248)	48.9 ± 8.2	
Self-reported inability to get chewed up food		
yes (n = 71)	42.7 ± 8.1	$< 0.05^b$
no (n = 230)	50.2 ± 7.8	
Missing teeth (25.2 ± 8.2)	48.4 ± 8.4	$< 0.05^c$
Decayed teeth (0.8 ± 1.9)	48.4 ± 8.4	$< 0.05^c$

[a]Kruskal-Wallis test; [b]Mann-Whitney U test; [c]*t*-test; TMJ – temporomandibular joint.

Table 5

Distribution of participants according to self-reported health-related ratings (n = 301)

Variable	n	%
Self-reported general health		
very bad	25	8.3
bad	122	40.5
good	133	44.2
very good	21	7.0
Self-reported oral health		
very bad	15	5.0
bad	89	29.6
good	182	60.5
very good	15	5.0
Self-reported need for dental treatment		
yes	135	44.9
no	166	55.1
Self-reported toothache		
yes	43	14.3
no	258	85.7
Self-reported sensitivity to hot and cold		
yes	62	20.6
no	239	79.4
Self-reported TMJ pain		
yes	20	6.6
no	281	93.4
Self-reported bleeding gums during brushing		
yes	34	11.3
no	267	88.7
Self-reported dry mouth		
yes	179	59.5
no	122	40.5
Self-reported bad breath		
yes	53	17.6
no	248	82.4
Self-reported inability to chew food		
yes	71	23.6
no	230	76.4

TMJ – temporomandibular joint.

Distribution of Geriatric Oral Health Assessment Index

The responses to the different questions of the GOHAI questionnaires are listed in Table 6. The GOHAI score of the examinees ranged from 23 to 60, with higher values indicating better quality of life. The average value of the GOHAI score was 48.4 (SD ± 8.4). As for the distribution of GOHAI answers, oral impacts were frequent for item 2: 48.5% of the participants reported 'always' having difficulties when chewing. On the other hand, a small number of participants (0.3%) used medications 'always' to relieve dental pain (item 8). Also, oral impacts were minimal for item 3: 0% of the participants answered with 'never' to the question 'How often were you able to swallow comfortably?'.

Discussion

This research shows that poor oral health of institutionalized elderly in Serbia has a negative impact on their daily activities and quality of life. The obtained results of psychometric characteristics of the Serbian GOHAI are satisfactory, with good reliability and convergent and concurrent validity. However, there are some issues about the applied methods and results that need to be discussed and compared to other studies.

The GOHAI was originally developed in English language and designed for use in North America. The quality of translation and validation of the translated instrument plays a significant role in ensuring that the results obtained in cross-cultural research are not due to errors in translation, but rather due to real differences or similarities between cultures in the measured phenomena [13, 14]. Considerable effort has been invested in the appropriate cultural adaptation to overcome language and cultural differences. The methods used (back translation and monolingual pre-testing) have been recommended by the WHO and experts in this field [12, 15], and proven to be valid in many studies carried out in different cultural settings. Also, to minimize possible negative effects and flaws of the back-translation method, in this research a preliminary version (after back translation) was pre-tested on a small sample of the target population (pilot study).

Considering the lower educational level in Serbia comparing to the countries of origin of the GOHAI, the forward method was used with simple phrases. Literal translation was avoided as much as possible, in accordance with recommendation of cross-cultural validation. The major problem was with question 5, mainly because of the positive "able to eat anything" and negative "without feeling discomfort" direction of this question. Because of that, some discrepancies

between the original text and back-translation were found, which were resolved by the research team. Additionally, problems of lexical compatibility occurred with the phrase "self-conscious" in question 10 because it does not have the dictionary equivalent in Serbian. Considering that the goal was semantics rather than literal equivalence [13, 16], an acceptable approximation was found (Appendix 1).

The frequency of examinees who gave positive answer to the question "How often were you able to swallow comfortably?" was very low. The same was noticed in a Dolan's longitudinal study [10]. Additionally, this question showed the low item-total and test-retest correlation, which is compatible with the results in the French version. This question was originally developed to detect problems with swallowing in the elderly, mainly because of xerostomia. Yet, the use of this question should be reconsidered as an instrument for the assessment of quality of life of younger populations in whom xerostomia is not commonly present.

The obtained values of the GOHAI score – mean 48.4 (SD ± 8.4) are larger than values in the Arabic version – mean 40.9 (SD ± 10.6) [17] and the French version – mean 46.4 (SD ± 9.5) [18] and very similar to the results of the Chinese version – mean 48.9 (SD ± 7.2) [19].

As far as the distribution of responses to certain issues, the negative impact of oral health on quality of life was observed in question 2. Almost every second participant always had trouble biting or chewing any kinds of food, such as firm meat or apples, due to problems with teeth or dentures, which is much more than in other studies (7.9%) [17]. A high percentage of patients with this response shows that half of the sample has problems in their daily diet and that their quality of life is poorer because of unsatisfactory oral health. For questions 3 and 8 there are no so many obvious discrepancies, compared with other studies.

Cronbach's alpha coefficient (0.79) confirmed a high degree of internal consistency and homogeneity among items. The value is similar to the Malay version of the GOHAI, and it is within the range of obtained values (0.64–0.88) of the coefficient of the GOHAI version in other languages.

The item-scale correlations varied from 0.24 to 0.56 in the Serbian version, compared with 0.28 to 0.61 in the Chinese version [19], -0.08 to 0.72 in the Romanian version [20], and 0.38 to 0.69 in the Malay version [21].

The obtained values for the test-retest correlation were within the range from 0.40 to 0.85, where the correlation coefficient for the GOHAI score was 0.66, which was similar to the values in the Swedish version (0.64) [22]. The lowest value was associated with the question "use of medication" with the correlation coefficient of 0.40.

Concurrent validity showed the relationship between the GOHAI score and the self-reported general and oral health and the self-reported need for dental treatment. Lower GOHAI scores were associated with lower ratings for self-reported general and oral health and self-reported need for dental treatment. However, the obtained values are lower than the values of the original English [7] and Arabic [18] versions of the GOHAI. Regarding limitations of concurrent validity, 60.5% of respondents thought that their present oral health status was satisfactory, and this value is larger than the values cited in other studies [21, 22]. This can be explained by the environment in which these old people lived. In this region (but also in many others) there is still the rule that for older age edentulousness is expected and the absence of pain is the equivalent to "good oral health." That is, there are different perceptions of what is "problematic" according to individual contexts, besides regional and historical tradition, where dental treatment is still poorly accessible, and where it will be more or less likely that a problem was interpreted or perceived as such [23]. Therefore, it is necessary to improve social importance of oral health and oral health care for institutionalized elderly. Consequently, over the years, this will change the expected image of "good oral health" in elderly, from a toothless person to person with natural teeth or dental prothesis.

Convergent validity confirmed the results of certain studies [18] by showing that self-reported toothache, sensitivity to hot and cold, dry mouth, bad breath and inability to chew food were related to the lower GOHAI scores. Additionally, the participants in the study with one or more missing or decayed teeth had lower GOHAI scores than those who did not have missing or decayed teeth.

Conclusion

The GOHAI final score was considered low, indicating low oral health self-perception by the institutionalized elderly in Serbia. These data suggest that oral health has a significant effect on the overall health of the institutionalized individuals.

The Serbian version of GOHAI showed acceptable reliability and validity for research of elderly. Future studies are necessary to determine the stability of the instrument as well as its sensitivity to dental treatment by correlating data between institutionalized and non-institutionalized persons.

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REFERENCES

1. *Sischo L, Broder HL.* Oral health-related quality of life: What, why, how, and future implications. J Dent Res 2011; 90(11): 1264−70.
2. *Mariko N, Tomohisa K, Wataru F, Megumi O, Michio Y, Nobuyuki H*, et al. Effects of dental treatment on the quality of life and activities of daily living ininstitutionalized elderly in Japan. Arch Gerontol Geriatr 2010; 50(1): 65−8.
3. *Yoon MN, Steele CM.* Health care professionals' perspectives on oral care for long-term care residents: Nursing staff, speech-language pathologists and dental hygienists. Gerodontology 2012; 29(2): e525−35.
4. *Erić J, Stančić I, Tihaček ŠL, Jelenković PA, Tsakos G.* Validity and reliability of the Oral Impacts on Daily Performance (OIDP) scale in the elderly population of Bosnia and Herzegovina. Gerodontology 2012; 29(2): e902−8.

5. *Slade GD, Spencer AJ*. Development and evaluation of the Oral Health Impact Profile. Community Dent Health 1994; 11(1): 3−11.
6. *Adulyanon S, Vourapukjaru J, Sheiham* A. Oral impacts affecting daily performance in a low dental disease Thai population. Community Dent Oral Epidemiol 1996; 24(6): 385−9.
7. *Atchinson KA, Dolan TA*. Development of the Geriatric Oral Health Assessment Index. J Dent Educ 1990; 54(11): 680−7.
8. *Matthias RE, Atchison KA, Schweitzer SO, Lubben JE, Mayer-Oakes A, Jong FD*. Comparisons between dentist ratings and self-ratings of dental appearance in an elderly population. Spec Care Dent 1993; 13(2): 53−60.
9. *Kressin NR, Atchison KA, Miller DR*. Comparing the Impact of Oral Disease in Two Populations of Older Adults: Application of the Geriatric Oral Health Assessment Index. J Public Health Dent 1997; 57(4): 224−32.
10. *Dolan TA*. The sensitivity of the geriatric oral health assessment index to dental care. J Dent Educ 1997; 6(1): 37−46.
11. *Walter SD, Eliasziw M, Donner A*. Sample size and optimal designs for reliability studies. Statist Med 1998; 17(1): 101−10.
12. *Kristjansson EA, Desrochers A, Zumbo B*. Translating and adapting measurement instruments for cross-linguistic and cross-cultural research: A guide for practitioners. Can J Nurs Res 2003; 35(2): 127−42.
13. *World Health Organization*. Oral health surveys: basic methods. 5th ed. Geneva: World Health Organization; 2013. (Arabic, Finnish, Portuguese)
14. *Maneesriwongul W, Dixon JK*. Instrument translation process: A methods review. J Adv Nurs 2004; 48(2): 175−86.
15. *Behling O, Law K*. Translating questionnaires and other research instruments: Problems and solutions. Thousand Oaks, CA: Sage Publications; 2000.
16. *Zumbo BD*. Validity as Contextualized and Pragmatic Explanation, and Its Implications for Validation Practice. In: *Lissitz RW*, editor. The Concept of Validity: Revisions, New Directions and Applications. Charlotte: IAP – Information Age Publishing; 2009. p. 65−82.
17. *Daradkeh S, Khader YS*. Translation and validation of the Arabic version of the Geriatric Oral Health Assessment Index (GOHAI). J Oral Sci 2008; 50(4): 453−9.
18. *Tubert-Jeannin S, Riordan PJ, Morel-Papernot A, Porcheray S, Saby-Collet S*. Validation of an oral health quality of life index (GOHAI) in France. Community Dent Oral Epidemiol 2003; 31(4): 275−84.
19. *Wong MC, Liu JK, Lo EC*. Translation and Validation of the Chinese Version of GOHAI. J Public Health Dent 2002; 62(2): 78−83.
20. *Murariu A, Hanganu C, Bobu L*. Evaluation of the Reliability of the Geriatric Oral Health Assessment Index (GOHAI) in Institutionalised Elderly in Romania: A Pilot Study. Oral Health Dent Manag 2010; 9(1): 11–5.
21. *Othman WW, Muttalib KA, Bakri R, Doss JG, Jaafar N, Salleh NC*, et al. Validation of the Geriatric Oral Health Assessment Index (GOHAI) in the Malay Language. J Public Health Dent 2006; 66(3): 199−204.
22. *Hagglin C, Berggren U, Lundgren J*. A Swedish version of the GOHAI index. Psychometric properties and validation. Swed Dent J 2005; 29(3): 113−24.
23. *Cornejo M, Pérez G, de Lima KC, Casals-Peidro E, Borrell C*. Oral Health-Related Quality of Life in institutionalized elderly in Barcelona (Spain). Med Oral Patol Oral Cir Bucal 2013; 18(2): e285−92.

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Appendix 1

Item responses (proportions) and items (n = 301)

During the past three month (Tokom poslednja tri meseca…)	Never (nikada)	Seldom (retko)	Sometimes (ponekad)	Often (često)	Always (uvek)
How often did you limit the kinds or amounts of food you eat because of problems with your teeth or dentures? (Koliko često ste ograničavali vrstu ili količinu hrane koju jedete zbog problema sa zubima ili protezama?)	174 (57.8%)	34 (11.3%)	28 (9.3%)	39 (13.0%)	26 (8.6%)
How often have you trouble biting or chewing any kinds of food, such as firm meat or apples? (Koliko često imate probleme sa odgrizanjem ili žvakanjem neke vrste hrane, kao što su žilavo meso ili jabuke?)	71 (23.6%)	22 (7.3%)	20 (6.6%)	42 (14.0%)	146 (48.5%)
How often were you able to swallow comfortably? (Koliko često ste mogli da gutate lagodno?)	0 (0%)	3 (1.0%)	9 (3.0%)	32 (10.6%)	257 (85.4)
How often have your teeth or dentures prevented you from speaking the way you wanted? (Koliko često su Vas Vaši zubi ili proteze sprečavali da govorite onako kako ste želeli?)	180 (59.8%)	28 (9.3%)	34 (11.3%)	30 (10.0%)	29 (9.6%)
How often were you able to eat anything without feeling discomfort? (Koliko često ste mogli da jedete bilo šta bez osećaja nelagodnosti?)	18 (6.0%)	31 (10.3%)	32 (10.6%)	53 (17.6%)	167 (55.5%)
How often did you limit contacts with people because of the condition of your teeth or dentures? (Koliko često ste ograničavali kontakt sa ljudima zbog stanja vaših zuba ili proteza?)	239 (79.4%)	15 (5.0%)	18 (6.0%)	22 (7.3%)	7 (2.3%)
How often were you pleased or happy with the looks of your teeth and gums, or dentures? (Koliko često ste bili zadovoljni ili srećni sa izgledom Vaših zuba i desni ili proteza?)	58 (19.3%)	33 (11.0%)	20 (6.6%)	56 (18.6%)	134 (44.5%)
How often did you use medication to relieve pain or discomfort from around your mouth? (Koliko često ste koristili lekove da bi ublažili bol ili nelagodnost u Vašim ustima?)	257 (85.4%)	13 (4.3%)	18 (6.0%)	12 (4.0%)	1 (0.3%)
How often were you worried or concerned about the problems of your teeth, gums or dentures? (Koliko često ste bili zabrinuti ili zaokupljeni problemima sa zubima, desnima ili protezama?)	150 (49.8%)	34 (11.3%)	27 (9.0%)	55 (18.3%)	35 (11.6%)
How often did you feel nervous or self-conscious because of problems with your teeth, gums, or dentures? (Koliko često ste se osećali nervozno zbog problema sa zubima, desnima ili protezama?)	172 (57.1%)	32 (10.6%)	30 (10.0%)	49 (16.3%)	18 (6.0%)
How often did you feel uncomfortable eating in front of people because of problems with your teeth or dentures? (Koliko često ste se osećali neprijatno da jedete pred ljudima zbog problema sa zubima ili protezama?)	214 (71.1%)	19 (6.3%)	29 (9.6%)	18 (6.0%)	21 (7.0%)
How often were your teeth or gums sensitive to hot, cold, or sweets? (Koliko često su Vam zubi ili desni bile osetljive na toplo, hladno ili slatko?)	217 (72.1%)	24 (8.0%)	26 (8.6%)	23 (7.6%)	11 (3.7%)

Geriatric Oral Health Assessment Index (GOHAI) score: mean 48.4 [standard deviation (SD) ± 8.4]; minimum = 23; maximum = 60.

ORIGINAL ARTICLE

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Joggle lap shear testing of deep occlusal composite restorations lined with Dycal, Dycal LC, conventional or resin-modified glass ionomer

Testiranje spojnog smicanja dubokih okluzalnih kompozitnih restauracija postavljenih preko podloge od Dycal, Dycal LC, konvencionalnog ili smolom modifikovanog glas-jonomer cementa

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Abstract

Background/Aim. The longevity of a dental restoration may be predicted to some degree by its adhesive ability, and this, in turn, can be measured by bond strength testing between restorative materials and tooth structure. The aim of this study was to test an innovative joggle lap shearing jig that integrates the tooth and the entire biomechanical unit into testing, to compare the shear bond strengths of Class I occlusal composite restorations in deep cavity preparations lined with Dycal, Dycal LC, conventional glass ionomer or resin-modified glass ionomer. The mode of failure (adhesive, cohesive, mixed) after debonding was determined by stereomicroscopy. **Methods.** A total of 150 standardized occlusal cavities were prepared and divided into five groups. The group I cavities (n = 30) were coated with adhesive (ExciTE®F) and filled directly with composite (TetricEvoCeram). The group II and III cavities were lined with Dycal (n = 30) or Dycal LC (n = 30) before placing composite. The groups IV and V specimens were based with Fuji IX (n = 30) or Fuji II LC (n = 30). Shear bond strengths were determined with a universal testing machine and fractured bonding sites were analyzed under stereomicroscope. The mean bond strengths were analyzed using one-way ANOVA test ($p < 0.05$) and the means between the groups were analyzed with Student's *t*-test. **Results.** The shear bond strength (MPa) of composite restorations in cavities without base (23.91 ± 4.54) was higher than cavities lined with Fuji II LC (17.45 ± 2.74), Fuji IX (8.76 ± 2.57), Dycal LC (13.07 ± 1.84) or Dycal (6.12 ± 1.28). The results using the jogged lap shearing jig were consistent with the literature. **Conclusion.** The shear bond strength of occlusal composite restorations in deep cavities without liners was greater than cavities lined with Fuji II LC > Fuji IX > Dycal LC > Dycal.

Key words:
biomechanics; dentin-bonding agents; adhesives; dental cements; calcium hydroxide; materials testing; in vitro.

Apstrakt

Uvod/Cilj. Trajnost zubnih nadoknada može se donekle predvideti vstom adhezivne sposobnosti materijala i može se meriti testiranjem snage adhezije restorativnih materijala i zubnih struktura. Cilj ove studije bio je da se da se testira preklapanje spoja koji povezuje zub i biomehaničku jedinicu i da se uporedi jačina veze okluzalnih kompozitnih ispuna postavljenih u duboke kavitete preko podloge od Dycal, Dycal LC, konvencionalnog ili smolom-modifikovanog glas-jonomer cementa. **Metode.** Ukupno 150 standardizovanih okluzalnih kaviteta bilo je podeljeno u pet grupa (n = 30): I – kaviteti premazani adhezivom (ExciTE®F) i direktno ispunjeni kompozitom (TetricEvoCeram); II i III – kaviteti sa podlogom od Dycal ili Dycal LC pre postavljanja kompozitnog materijala; IV i V – uzorci sa bazom od Fuji IX ili Fuji II LC (n = 30). Jačina vezivne snage određena je pomoću univerzalne mašine, a način neuspeha (adhezivna, kohezivna, mešovita fraktura) određen je stereomikroskopom. Srednje vrednosti su analizirane pomoću ANOVA testa ($p < 0,05$), a značajnost razlika između grupa analizirana je Student-ovim *t*-testom. **Rezultati.** Jačina vezivne snage (MPa) kompozitnih ispuna u kavitetima bez podloge (23,91 ± 4,54) bila je veća u poređenju sa ispunima postavljenim preko Fuji II LC (17,45 ± 2,74), Fuji IX (8,76 ± 2,57), Dycal LC (13,07 ± 1,84) ili Dycal (6.12 ± 1.28). **Zaključak.** Smicanje ili pomaknuće okluzalnih kompozitnih ispuna u dubokim kavitetima bez lajnera je veće nego u kavitetima postavljenim preko Fuji II LC > Fuji IX > Dycal LC > Dycal.

Ključne reči:
biomehanika; dentin, vezivna sredstva; adhezivi; zub, cement; kalcijum hidroksid; materijali, testiranje; in vitro.

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Introduction

The longevity of a dental restoration may be predicted to some degree by its adhesive ability, and this, in turn, can be measured by bond strength testing. However, bond strength values are at best, gross assessments of the efficacy of bonding restorative materials to dentin as there is no direct clinical correlation to predict their clinical performance [1–4]. The International Standards Organization (ISO) Technical Specification No. 11405 provides some guidelines for testing the adhesive bond between restorative materials and tooth structure [5]. However, there is currently no universal test that will accurately predict the clinical performance of a specific material [6, 7].

Macro-bond strengths can be measured by shear, tensile, or push-out tests [8]. In a shear bond test, two materials are connected *via* an adhesive agent and loaded in shear until fracture occurs. Though macrotests are known for their simplicity, they have their shortcomings [7, 9]. Factors influencing bond strength testing include issues related to the dentin substrate, composite, and bonding area [10], storage conditions of the bond assemblies, and test design [11]. Another source of variability is the method used to apply the shear force, which includes wire loops, points, and knife edges [12].

In tensile bond tests, load will be exerted on either side of the test specimen, which can be held by active or passive gripping methods [13]. Stresses are far more homogeneous across the interface than in shear and, therefore, maximum principal stress values are much closer to the nominal strength [9, 14]. To pull a bond, however, requires the substrate and interconnect to be gripped. In these cases, a set of accurately formed and aligned tweezer tips with precision control of their opening and closing is likely to make the difference between success and failure [1]. Specimen alignment is also critical to avoid uneven stress distribution upon the specimen during loading.

Shear bond strengths highlight the strength at the bonded interface. When the shear device applies load forces on larger contact areas such as with the wire loop [15], stainless steel tape [16] and the Ultradent device [17], higher shear bond strength values are expected due to a more even distribution of shear forces. Nevertheless, specimen size [18], loading length [19], adhesive layer thickness [20], loading site [17] have been reported to affect the bond strength values and failure modes. In comparison to a knife edge, the use of a wire-loop seems to reduce the stress concentration magnitude adjacent to the adhesive interface, but finite element analysis has shown that this method results in grossly underestimated bond strength values [12].

Microtensile bond testing has also been used widely to assess bond strengths. However, the method is not easily applied to enamel due to its brittleness and the stresses generated during specimen preparation can lead to fracture of the enamel-resin interface [15]. The lack of standardization among microshear bond testing studies has resulted in considerable discrepancies in bonding data [21]. Furthermore, microtensile bond testing is also subject to the influence of cross-section shape and surface area, [22, 23] cutting speed [24], and geometry of the specimens, as well as the mode of fixation and the devices used for testing [25]. The shear load must also be applied precisely at the bonded interface to avoid subjecting the cylinder to rotation or bending rather than shear tension [21].

A relatively thicker adhesive layer in microshear tests, among other reasons, concentrates stresses highly influencing the maximum load, thereby rendering microtensile tests less accurate than macrosshear tests in representing shear bond strength [1]. Moreover, none of these laboratory tests take into account the overall response of a restored tooth while being loaded under the same shear forces, as the tests focus specifically on the adhesive layer between the tooth substrate and restorative material and not on the whole biomechanical unit.

Normal tooth structure transfers external biting loads through enamel into dentin as compression, which are distributed over a large internal volume of tooth structure and thus local stresses are lower. A restored tooth tends to transfer stress differently than an intact tooth, and in turn, differently from cross-sections of teeth that are subject to evaluation by current testing methods. Any force on the restoration produces compression, tension or shear along the tooth/restoration interface, leading to complex stress distributions, a combination of compressive, tensile and shear stresses (Figure 1) [26]. Since the process of mastication is one of indentation, basically a shearing phenomenon, the true nature of adhesive strength of the materials at the interface is depicted by the shear bond strength.

Ultimately, the quality and efficacy of bonding of adhesive materials will be reflected in their mode of failure – either cohesive, adhesive or mixed. With increasing bond strengths, the number of cohesive failures within the dentinal substrates is expected to increase [26]. A more ideal clinical correlation, however, would consider the whole biomechanical unit which includes not only the restorative material, but the tooth structure and the interface between the restoration and the tooth, as well. The restorative material may be strong enough to resist fracture, but the interface or tooth structure may not be.

Most restorations are designed to distribute stresses onto sound dentin, rather than to enamel. The process of stress transfer to dentin becomes more complicated when the amount of remaining dentin is thin and the restoration must bridge a significant distance to seat on to thicker dentin with the use of liners and bases. A test that relates the line of action of shear force more directly to the adhesive layer as it occurs within the tooth and to the actual restoration would more realistically depict how the material would resist debonding under shear forces during mastication.

Therefore, the aim of the study was to test an innovative joggle lap shear testing jig compare the shear bond strengths of class I occlusal composite restorations in deep cavity preparations lined with Dycal, Dycal LC, conventional glass ionomer or resin-modified glass ionomer, by centering the line of action of the applied shear force at the location of the dentin-liner or dentin-adhesive layer as it occurs in the tooth and within an actual restoration. The specified liners were chosen for this study, as the literature contains an abundance of data

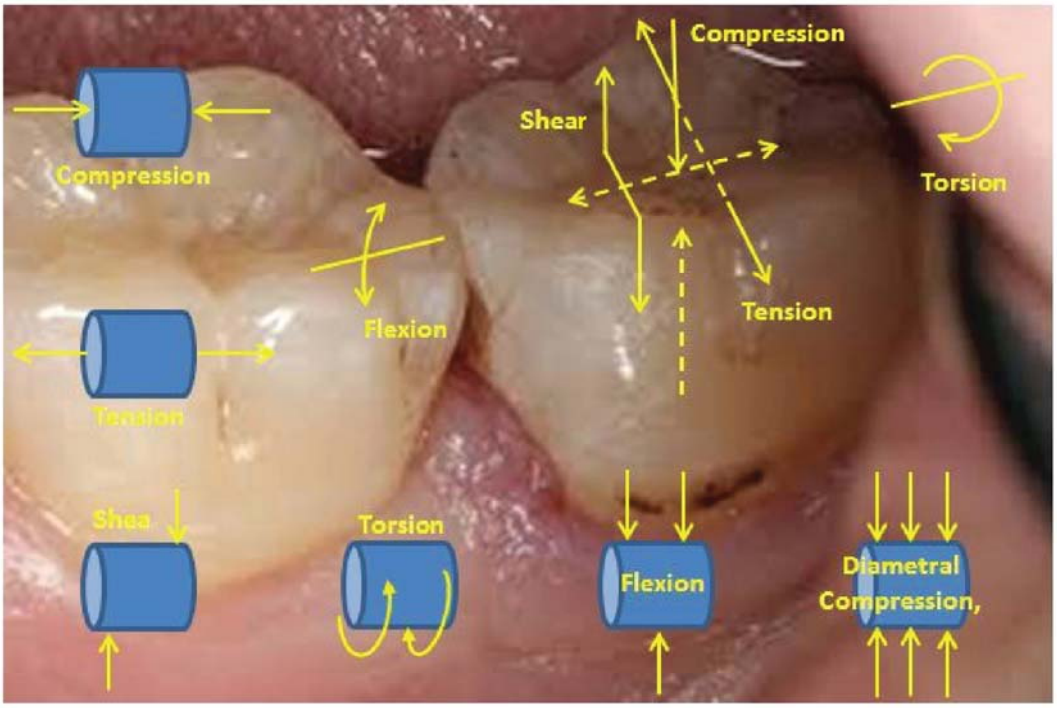

Fig. 1 – Normal tooth structure transfers external biting loads through enamel into dentin as compression, which are distributed over a large internal volume of tooth structure. A restored tooth tends to transfer stress differently than an intact tooth, and any force on the restoration produces compression, tension or shear along the tooth/restoration interface, leading to complex stress distributions; a combination of compressive, tensile and shear stresses.

related to their shear bond strengths to serve as the basis for comparison.

Methods

This *in vitro* study was approved by the Macedonian Ministry of Health Research and Ethics Committee and the Institutional Review Board of Sts. Cyril and Methodius University. A total of 150 non-carious mandibular third molars, extracted for orthodontic reasons with similar crown size were selected, cleaned and stored in a solution of 0.5% chloramine-T at 4°C until used. Bond strengths were measured no more than six months *post* extraction as *per* ISO technical specification 11405 [5].

To ensure that the teeth were free of cracks, defects or caries they were examined at × 10 magnification by means of an optical microscope (SZ-TP Olympus; Tokyo, Japan). To facilitate cavity preparation, the cusp tips of each tooth were reduced with a double-faced diamond disc No. 7011 (KGSorensen Ind. Com. Ltd), producing a flattened occlusal dentin surface. The prepared dentin surfaces were then polished with 180, 320, and 600 grit wet silicon carbide paper for 60 s.

Standardized cavity preparations were created in each tooth by mounting each specimen in plaster within a metal mold and using a variable-speed electric drill (Dremel, Model 232-5, Emerson Electric Co, Racine, WI) mounted on a drill press apparatus to facilitate uniform preparation of cavities and accurate cavity depth dimensions [27]. The occlusal cavity preparations, 3 mm (length) × 3 mm (width) × 3.0 mm (depth), followed a rectangular outline drawn on the occlusal surface of the tooth and were made with a #110/010 diamond bur (Dentsply, York, PA, USA). Cold water spray was delivered to the tooth and bur during cavity preparation to minimize heat. To standardize surface roughness, a new diamond bur was used for each preparation.

The teeth were then randomly divided into 5 groups *per* 30 teeth, each based on the restorative materials tested as follows: the group I (control group) specimens (n = 30) were treated with a complete adhesive system: etched with 37% H_3PO_4, coated with ExciTE® adhesive (IvoclarVivadent, Amherst, NY, USA) and filled directly with TetricEvoCeram composite (IvoclarVivadent). Group II and III specimens were first lined with either self-cured Dycal (Dentsply) (n = 30) or light-cured Prisma® VLC Dycal® (Dycal LC) (Dentsply) (n = 30) calcium hydroxide liners, respectively, then etched, coated with ExciTE adhesive and filled with Tetric Evo Ceram composite. Group IV and V specimens were first lined with either conventional Fuji IX (GC, Tokyo, Japan) (n = 30) or resin modified Fuji II LC glass ionomer (GC Fuji LINING LC PASTE PAK; GC, Tokyo, Japan) (n = 30), then etched, coated with adhesive and filled with composite.

After the cavity preparations were filled according to the manufacturer's recommendations (Table 1), tygon tubing was attached to the occlusal surface of each restoration and filled with composite. The tygon tube was removed after curing, resulting in cylinders of resin composite with cross-sectional diameter and height of 3 mm respectively.

Table 1

Materials and Application Protocols

Material	Application Protocol
Dycal	Equal volumes of the base and catalyst paste (1.17 g:1.00 g) were extruded onto a mixing pad and stirred immediately using a Dycal applicator for 10 sec until a uniform color was achieved. After drying the cavity preparation, the mix was placed in the cavity with the Dycal applicator and spread over the floor to a depth of 1 mm before setting starts (setting time: 2 ½–3 ½ min at room temperature). Any excess set material was removed from margins with a sharp spoon excavator.
Prisma® VLC Dycal® Visible Light Cured Calcium Hydroxide Base/Liner	The cavity preparations were rinsed with water and gently dried with a cotton pellet to avoid dessication. The Prisma® VLC Dycal® Liner was dispensed on a parchment paper pad. Using a ball-pointed Dycal® Liner applicator, the Prisma® VLC Dycal® Liner was placed directly only on the deepest portion of the cavity dentin in a thin layer, not exceeding a thickness of 0.8–1 mm. Care was taken to avoid placing Prisma® VLC Dycal® Liner on enamel or the margins of the cavity, leaving the rest of the cavity surface free for bonding. The ball of the instrument is approximately 0.7 mm in diameter which can be used as an indicator for the thickness of the material being placed. The material was light cured at 470 nm, with minimum light output at least 300 mW/cm^2 exposure for at least 20 s. Any material excess from retention areas, enamel, and/or margins was removed with a sharp spoon excavator. The adhesive and restoration was then placed into the cavity preparation following manufacturer's directions.
GC Fuji LINING LC PASTE PAK	GC Fuji Lining LC Paste Pak is a radiopaque, light cured resin-modified glass ionomer lining cement available in paste-paste form. The Paste Pak cartridge was loaded into the Paste Pak Dispenser after sitting at room temperature for 30 min. The cartridge was bled in order to prevent the incorporation of air bubbles into the material. After dispensing onto a mixing pad, the material was incorporated and spread out in a thin layer on the mixing pad using a plastic spatula. The pastes were mixed thoroughly, with lapping strokes, for 10 seconds, with care not to incorporate air bubbles. The working time is 2 minutes 15 s from the start of mixing at 23°C (73.4°F). The tooth preparations were washed and dried but not dessicated. The cement was transfered to the preparation using a syringe, covering dentine up to a depth of 1 mm, and light cured with a halogen light curing device which was placed as closely as possible to the cement surface for 20 sec.
ExciTE® F	ExciTE® F is a light-curing, nanofilled, fluoride-releasing, single-component adhesive for dentin and enamel bonding in conjunction with the total-etch technique. After ensuring a dry operating field, areas in deep cavities close to the pulp were selectively coated with a calcium hydroxide liner. A 37% phosphoric acid gel was applied (Total Etch, IvoclarVivadent) to the prepared enamel and flowed onto the prepared dentin. The etchant was left to react on the enamel for 15–30 s and on the dentin for 10–15 s. Following this, all etchant gel was removed with a vigorous water spray for at least 5 s. Excess moisture was removed with an air gun, leaving the dentin surface with a glossy wet appearance (wet bonding) not to over dry the dentin. The first step in applying Excite is to etch the enamel for 15 s and the dentin for 10 seconds with Total Etch, a 37% phosphoric acid etchant. The etchant is removed with thorough rinsing and the tooth structure is lightly dried with air or blot dried. Excite Adhesive is generously applied to the tooth structure using a scrubbing motion for 10 s, gently air dried for 3 seconds, and light activated for 20 s. The restoration is then placed using standard techniques.
TetricEvoCeram	TetricEvoCeram is a light-curing, radiopaque, nanohybrid composite for direct restorative therapy. The cavity preparations were cleaned and carried out according to the requirements of the adhesive technique with care to avoid preparing sharp, internal edges or additional undercuts. Any sharp enamel edges were rounded with finishing diamonds (25–40 μm). Subsequently, all residue in the cavity was removed with water spray and dried with water- and oil-free air. Only very deep areas close to the pulp with a calcium hydroxide material with care not to cover other cavity walls, since they can be used to support the bond with an enamel/dentin adhesive. Conditioning and application of the bonding agent was performed according to the Instructions and recommendations of the manufacturer using ExciTE® F (with phosphoric acid etching) . TetricEvoCeram was applied at room temperature in layers of max 2 mm thickness and adapted with a suitable instrument (e.g., OptraSculpt). Excess material was removed with suitable finishers (e.g., Astropol® F) or fine diamonds after polymerization. TetricEvoCeram was cured with light in the wavelength range of 400–500 nm (blue light) with a high intensity quartz tungsten halogen lamp (Astralis 10, (IvoclarVivadent) at High Power Program Regime, at 40 s exposure time, and 1200 mW/cm^2 light intensity, holding the tip of the light (8 mm) about 3mm above the restoration.

All the specimens were then thermocycled 500 times at 5°C and 55°C water with a one-minute dwell time. The specimens were then stored in distilled water for 24 h at 37°C to simulate the conditions of the oral cavity and subjected to shear bond strength testing in a universal testing machine (ADMET eXpert 1000 servo-hydraulic mechanical testing machine, ADMET, Norwood, MA) at a crosshead speed of 0.5 mm/min. The shear bond strenght megapascals (MPa) was calculated as ratio of maximum load recorded at failure in Newtons to surface area of the bonded restorations in mm^2.

The specimens were arranged in the mounting jigs of the testing machine. An *ad hoc* assembly of two vertical joggled lap metal holders was manufactured with holes on the ends to serve as holders for all dental elements. With the bevels parallel and facing away from each other, each tooth specimen was locked into one holder, while the composite button on the occlusal surface of the restoration engaged a 3 mm hole that was machined in the other holder. After fixing both tooth and button in place with orthodontic resin, the samples were subjected to shear force (plane stress) on the adhesive interface by stretching vertically on the specimen from both directions until the restorations failed (Figure 2).

Self-tightening vise grips were used to prevent slipping. The metal holders were aligned so that the centerline of the grip assembly was aligned with the adhesive bond at the bottom of the cavity preparations. Proper alignment was achieved with vice grips by adjusting the grip inserts from side to side so that the center line of the upper and lower grips passed through the dentin-adhesive layer. The teeth were then fixed to the metal holders with orthodontic resin to provide additional retention to avoid any sliding during the tests. All the procedures were conducted at room temperature.

Shear force was applied to each specimen by the servo-hydraulic mechanical testing machine (ADMET, Norwood, MA, USA) at the crosshead speed of 0.5 mm/min until failure occurred. Shear bond strength was then calculated in units of MPa after measuring the cross-sectional area at the site of fracture according to the formula: $\tau = F/A$ ($N/mm^2 = MPa$). Mean shear bond strengths and standard deviations were calculated for each group and statistically analyzed using a one-way ANOVA analysis of variance ($p < 0.05$). Comparison of the means between the groups was conducted with Student's *t*- test.

Failure modes were evaluated by a single operator under a dissecting microscope (SZ-TP Olympus; Tokyo, Japan) and classified as: adhesive failure (occurring purely at the restoration-dentin interface); cohesive failure (occurring within the material or within dentin); mixed adhesive/cohesive failure (combination of the adhesive or any of the cohesive modes). Calcium hydroxide and glass ionomer cements had been leveled off flush with the surface of the cavity preparation prior to the composite resin placement. Any deficiency in the cement surface was categorized as a cohesive failure within the material. If the surface of the two materials remained flat, this represented adhesive failure of the bond, and positive elevations on the cement surface represented a cohesive failure in the composite resin.

Schematic representation of biting loadstransfer is given in Figure 3.

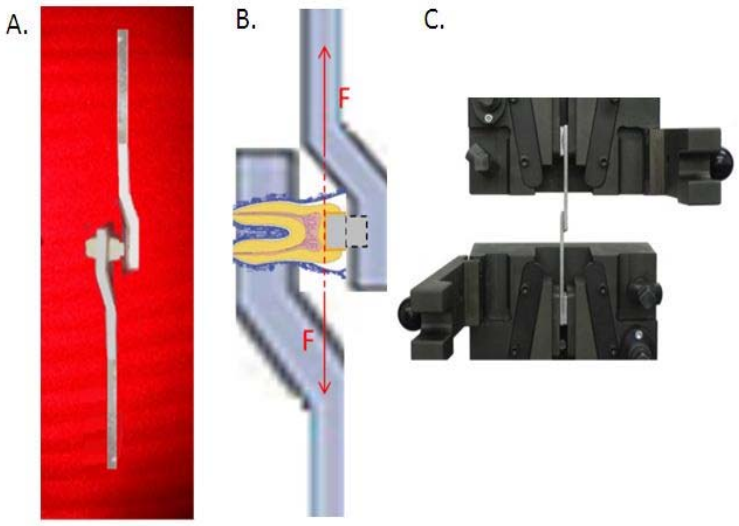

Fig. 2 – (A) An *ad hoc* assembly of two vertical joggled lap metal holders with holes on the ends serve as holders for all dental elements. With the bevels parallel and facing away from each other, each tooth specimen is locked into one holder, while the composite button on the occlusal surface of the restoration engages a 3 mm hole that is machined in the other holder. (B) After fixing both the tooth and the button in place with orthodontic resin, the samples were subjected to a shear force (plane stress) on the adhesive interface by stretching vertically on the specimen from both directions until the restorations failed. (C) Self-tightening vise grips were used to prevent slipping. The metal holders were aligned so that the centerline of the grip assembly was aligned with the adhesive bond at the bottom of the cavity preparations. Proper alignment was achieved with vice grips by adjusting the grip inserts from side to side so that the center line of the upper and lower grips passed through the dentin-adhesive layer. The teeth were then fixed to the metal holders with orthodontic resin to provide additional retention to avoid any sliding during the tests.

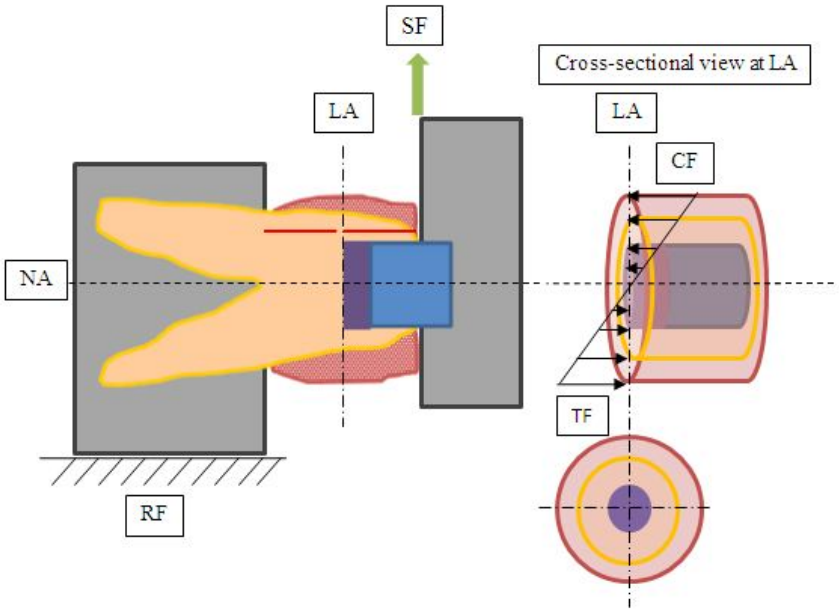

Fig. 3 – Applying tensile force accurately along the plane gives rise to configurations that minimise distortion away from the plane. Each end of the sample is held by vice grips and pulled apart at the controlled rate, and the force applied is expressed proportionally to the total adhesive surface area, or shear area. The joggled lap metal holders suspend the restored teeth bucco-lingually in a horizontal position and center the line of action to the specified adhesive layer as it relates to the restored tooth, while two fulcrums create a moment and subsequent torque to more closely simulate load under lateral excursions during mastication.
Red area – Enamel; Yellow area – Dentin; Blue area – Composite; Purple area – Baseliner; Gray areas –Test fixtures; RF – Rigid fixture (not moving); SF – Shear force application moving fixture; CF – Compression internal-forces; TF – Tension internal forces; LA – Line of action; NA – Neutral axis.

Results

The mean and standard deviation of shear bond strength for each group are presented in Table 2. The shear bond strength of composite restorations in cavities filled directly with complete adhesive system (37% H_3PO_4 etch, ExciTE adhesive and TetricEvoCeram composite) was higher than the shear bond strength of restorations in cavities lined with either of the calcium hydroxide bases (Dycal and Dycal LC) or glass ionomer cements (Fuji IX and Fuji II LC) (Figure 2). The difference in mean shear bond strength values between the control group and cavities lined with either calcium hydroxide or glass ionomer bases was significant ($p < 0.001$). The adhesive strength of restorations bonded directly to dentin-enamel was approximately four times greater than cavities lined with conventional Dycal and twice as high as the light-cured Dycal group. The adhesive strength of restorations in the control group was 2.7 times greater than the conventional glass ionomer group and 1.4 times greater than the resin-modified glass ionomer group.

Comparison of mean shear bond strengths between composite restorations in cavities lined with Dycal or Dycal LC (Table 2) showed significant differences ($p < 0.001$). The shear bond strength of the restoration over light-cured Dycal was twice (213.58%) as strong or 113.5% better than with conventional Dycal (6.12 MPa vs 13.07 MPa), though half as strong as composite bonded directly to hard tooth structure (13.07 MPa *vs* 23.91 MPa).

The differences in the mean shear bond strength values between composite restorations in the group III and IV cavities lined with Fuji IX or Fuji II LC glass ionomer were also significant ($p < 0.001$). The strength of adhesion of composite res-

Table 2

Comparative analysis among groups

Group		Average shear bond strength (MPa)	Standard deviation (SD)	Student's *t*-test	ANOVA F-test	*p*
I	Control	23.91	4.54			
II	Dycal	6.12	1.28	11.827	139.91	< 0.001
III	Dycal LC	13.07	1.84	7.197	52.04	< 0.001
IV	Fuji IX	8.76	2.57	9.732	92.33	< 0.001
V	Fuji II LC	17.45	2.74	4.113	18.22	< 0.001

torations over modified resin glass ionomer was two times (199.2%) greater than conventional glass ionomer and 33.5% greater than light-cured Dycal (13.07 MPa *vs* 17.45 MPa).

The resin-modified glass ionomer lined composite restorations reached higher shear bond strengths than the remaining liner groups. The GC Fuji LC cement showed superior bond strengths than all the other materials tested, except for the control group which had notably higher mean shear bond strength. Composite restorations in cavities lined with conventional Dycal showed the lowest shear bond strength rate and were inferior to resin-modified cement.

The distribution of fracture modes observed with a dissecting microscope × 20 magnification is shown for all the groups in Table 3. The principle mode of failure in group I (control group) was adhesive at the composite-dentin interface. The mode of failure in the groups II and III was mainly cohesive, principally at the composite resin-Dycal or Dycal LC interface. The failures within the Fuji IX glass ionomer group IV were mainly cohesive, unlike the GC Fuji Lining LC group V which showed 50% adhesive failure. Superior results were observed with resin-modified cement (17.23 MPa). The failure observed was mainly within the cement.

In lap shear (tensile) testing, adhesion is tested by pulling bonded layers apart along the plane of adhesion. The result can be a clean breakaway of the adhesive layer from the substrate, or more likely a breakdown in the cohesion of either the substrate or the adhesive layer, or both. Applying tensile force accurately along the plane gives rise to configurations that minimize distortion away from the plane. Each end of the sample is held by vice grips and pulled apart at a controlled rate, and the force applied is expressed proportionally to the total adhesive surface area, or shear area. In this study, jogged lap metal holders suspended the restored teeth buccolingually in a horizontal position and centered the line of action to the specified adhesive layer as it relates to the restored tooth, while two fulcrums created a moment and subsequent torque to more closely simulated load under lateral excursions during mastication.

The offset jig fixture allows for the entire composite cylinder to fit completely into the metal holder and flush with the restoration's occlusal surface. The opposite fixture mounted the tooth in a configuration that mimics the emergence of the cementoenamel junction (CEJ) from the alveolus. With both fixtures separated an equal distance from the

Table 3

Failure modes of all test groups

Description	Adhesive (%)	Cohesive (%)	Mixed (%)
Control (Excite + Tetric Ceram)	100		
Dycal + (Excite + Tetric Ceram)		80	20
Dycal LC + (Excite + Tetric Ceram)		80	20
Fuji IX + (Excite + Tetric Ceram)	10	70	20
Fuji II LC + (Excite + Tetric Ceram)	50	20	30

Discussion

As many variables can affect the efficacy of any shear bond strength testing method, it is important to justify the experimental model. The choice of testing assembly has great influence on stress distribution. Traditional shear testing with a knife or round surface contact have several limitations, including high stress point loading in which load is concentrated and not distributed to the surrounding composite or natural tissue structures. Furthermore, knife-edge shearing can only produce compression loading from the contact force. The use of a knife-edge chisel causes more severe stress concentration at the load application area than wire loop [9, 12] and stainless steel tape allows more uniform stress distribution at the bond interface [28]. The distance between the point of load application and the bonded interface in shear tests also affects stress distribution [18].

In contrast, the novel shear test distributes loading throughout the composite and tooth as it incorporates into testing the entire biomechanical unit. Distributed loading produces tension and compression loading within the entire composite, which is integrated into material and tissue structures. This was achieved by aligning the line of action to the floor of the restoration which may more closely simulate shear as seen clinically during mastication.

restoration floor, the weakest point in the restoration, the line of action being centered the novel test produces a shear and moment force coupled with tension and compression forces.

The viability of this jig was then tested by measuring bond strengths of a composite restoration in deep occlusal cavities lined with different bases. These studies have been limited. Therefore, a comparative *in vitro* analysis of composite restorations in deep occlusal cavity preparations lined with self-cured and light-cured CaOH and conventional and resin-modified glass ionomer would also be of further value [29, 30].

Many authors have measured shear bond strength *in vitro* with values ranging from 13.7 MPa to 26.84 MPa [31]. Khatry et al. [32] found mean values of adhesion to hard dental tissue with conventional composite and nanocomposite to be 21.04 MPa and 20.78 MPa, respectively. In general, they all show that maximum adhesive strength is achieved with direct bonding between hard dental tissues and adhesive systems [33, 34]. The current study determined that mean shear bond strength of a composite restoration bonded directly to dentin in occlusal cavity preparations (group I) was 23.91 MPa, which is consistent with the literature.

Sano et al. [22] reported that for specimens with rectangular bonding areas between 0.25–11.65 mm^2, tensile bond strength to dentin was shown to decrease as bonding area in-

creased, following a logarithmic function. A similar trend was noticed in shear bond strengths where smaller surface areas had significantly higher values when compared with those of larger areas [35]. The ISO/TR 11405 does not identify a specific value for bond area but it mentions a clear delimitation of the bonding area as an important requirement and shows a diagram of a split mold with a 3-mm diameter hole.[5] When considering that the resin composite in this study was bonded to four axial walls as well as the dentinal floor with an area of 9 mm^2 and that the shear bond strength was still in the upper end of values reported in the literature, our results cannot be correlated to the conclusions of the Sano et al. study [22].

Thermal cycling has been used as a technique to simulate clinical conditions and was used in this study as well. Thermal cycling may not have a significant effect on shear bond strength, but it can lead to spontaneous debonding of specimens and significantly reduce the shear bond strengths of dentin [36–38]. Miyazaki et al. [39] found that dentin bond strengths significantly decreased after 30,000 thermal cycles. A short thermal cycling regimen of 500 cycles was therefore, used in this study as recommended by ISO-TR 11450 [5].

Other investigations have found that the shear bond strength can be affected by the shrinkage of the composite material, which causes separation of the composite material from the dentin and, consequently, results in microleakage [40, 41]. According to Davidson et al., [42] the strength after polymerization contraction on a three-dimensional model is about 20 MPa. Munksgaard et al. [43] reported that a shear bond strength of 17 MPa was enough to counter shrinkage during composite polymerization to maintain the bonded interface.

Using liners in deep cavities can further affect the adhesion between hard dental tissues and composite materials [44]. Although calcium hydroxide as a base has desirable antibacterial effects and protects the pulpal tissue [33], it does not have notable adhesion capacity [30, 45]. Light-cured calcium hydroxide, on the other hand, has better chemical properties compared to self-cured calcium hydroxide and some investigators have reported good composite adhesion over Dycal LC [46]. The current study applied both Dycal and Dycal LC to the dentin of cavity preparations and found that the strength of adhesion of Dycal LC was significantly higher ($p < 0.001$).

The study determined that the shear bond strength of a composite resin over conventional glass ionomer Fuji IX or resin-modified glass ionomer Fuji II LC in an occlusal restoration was 8.76 MPa and 17.45 MPa, respectively. Although the mean shear bond strength of the light-cured Dycal group was twice (213.58%) as strong and 113.5% better than the conventional Dycal group (6.12 MPa *vs* 13.07 MPa), the strength of adhesion with modified resin glass ionomer was two times (199.2%) greater than conventional glass ionomer and 33.5% greater than light-cured Dycal (13.07 MPa *vs* 17.45 MPa). Assuming that the minimum strength of adhesion of a composite restoration to the cavity must be 17 MPa to maintain a good bond [43], the strength of adhesion achieved with modified resin glass ionomer equalled or slightly surpassed the minimum requirements. The results observed with resin-modified cement (17.23 MPa) are probably due to the superior cohesive strength of the cement and due to the chemical bonding between the resin bonding agent and the non-reacted resinous phase of the glass ionomer cement. The study indicates that a composite restoration in a deep occlusal cavity lined with modified resin glass ionomer would resist shear forces better than a composite restoration in a cavity lined with conventional glass ionomer or both self-cured and light-cured calcium hydroxide liners.

These results concur with other investigators who have explored the strength of adhesion between conventional and LC glass ionomer and have found that LC glass ionomers demonstrate better adhesion [47–50]. In a retrospective clinical study comparing direct composite materials with an indirect sandwich technique using resin modified glass ionomer as a base, Opdam et al. [51] found that direct composite restorations lasted longer. This might be due to resin-modified glass ionomer penetrating demineralized dentin better than conventional glass ionomer [34].

Nonetheless, both calcium hydroxide and glass ionomer liners can reduce the bonding surface available to composites, which can further contribute to reduced bond strength [29, 52–54]. Although the current study found that composite restorations in deep occlusal cavities lined with Fuji II LC resisted debonding better than the composite restorations over the other tested liners, the strength of the bond was not ideal.

As with any alternative test, the results of the *in vitro* study cannot be extrapolated directly to clinical situations. The complex intraoral environment prevents perfect duplication in *in vitro* conditions. Nevertheless, the *in vitro* information has to be considered along with the fact that to date, no single testing condition *in vitro* has proven superior over any other. The results of this study are comparable with the results achieved by other testing methods and show that the joggled shearing jig is a viable option that merits further investigation. It also provides a model that takes into account the whole biomechanical unit during testing.

Conclusion

The study shows that joggled lap shear testing may be a viable tool to measure strengths of dental materials while integrating the entire biomechanical unit into testing. A composite restoration in deep cavities lined with modified resin glass ionomer resisted debonding better than cavities lined with conventional glass ionomer or both self-cured and light-cured calcium hydroxide liners, satisfying a minimum adhesive strength of 17 MPa often cited as required to maintain composite marginal integrity. Shear bond strength of occlusal composite restorations in deep cavities without liners as measured by a joggled lap shear testing jig was greater than cavities lined with Fuji II LC, Fuji IX, Dycal LC and Dycal. The results concur with studies using other testing methods. The resin-modified glass ionomer lined composite restorations reached higher shear bond strengths than the remaining liner groups. The GC Fuji LC cement showed superior bond strengths than all the other materials tested, except for the control group which had notably higher mean shear bond strength. Composite restorations in cavities lined with conventional Dycal showed the lowest shear bond strength rate and were inferior to resin-modified cement.

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Disclosure statement

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Sirisha K, Rambabu T, Shankar YR, Ravikumar P.* Validity of bond strength tests: A critical review. Part I. J Conserv Dent 2014; 17(4): 305−11.
2. *Sudsangiam S, Noort R.* Do dentin bond strength tests serve a useful purpose. J Adhes Dent 1999; 1(1): 57−67.
3. *Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P*, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003; 28(3): 215−35.
4. *Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B.* Clinical effectiveness of contemporary adhesives: A systematic review of current clinical trials. Dent Mater 2005; 21(9): 864−81.
5. Dental materials: Testing of adhesion to tooth structure. ISO/TS 11405:2003 [published on 2003 February 1]. Available from: www.iso.org/iso/catalogue_detail.htm?csnumber...
6. *Heintz SD.* Systematic reviews: I. The correlation between laboratory tests on marginal quality and bond strength. II. The correlation between marginal quality and clinical outcome. J Adhes Dent 2007; 9 Suppl 1: 77−106.
7. *Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A*, et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010; 26(2): e100−21.
8. *Salz U, Bock T.* Testing adhesion of direct restoratives to dental hard tissue - a review. J Adhes Dent 2010; 12(5): 343−71.
9. *Braga RR, Meira JB, Boaro LC, Xavier TA.* Adhesion to tooth structure: A critical review of "macro" test methods. Dent Mater 2010; 26(2): e38−49.
10. *De Munck J, Mine A, Poitevin A, Van Ende A, Van Meerbeek G.* Testing bond strength. A review of the literature. Dent Mater 2010; 26: e139−40.
11. *Leloup G, Hoore DW, Bouter D, Degrange M, Vreven J.* Meta-analytical review of factors involved in dentin adherence. J Dent Res 2001; 80(7): 1605−14.
12. *DeHoff PH, Anusavice KJ, Wang Z.* Three-dimensional finite element analysis of the shear bond test. Dent Mater 1995; 11(2): 126−31.
13. *Armstrong S, Geraldeli S, Maia R, Raposo LH, Soares CJ, Yamagawa J.* Adhesion to tooth structure: A critical review of "micro" bond strength test methods. Dent Mater 2010; 26(2): e50−62.
14. *Van Noort R, Noroozi S, Howard IC, Cardew G.* A critique of bond strength measurements. J Dent 1989; 17(2): 61−7.
15. *Foong J, Lee K, Nguyen C, Tang G, Austin D, Ch'ng C*, et al. Comparison of microshear bond strengths of four self-etching bonding systems to enamel using two test methods. Aust Dent J 2006; 51(3): 252−7.
16. *Sinhoreti MA, Consani S, De Goes MF, Sobrinho LC, Knowles JC.* Influence of loading types on the shear strength of the dentin-resin interface bonding. J Mater Sci Mater Med 2001; 12(1): 39−44.
17. *Pecora N, Yaman P, Dennison J, Herrero A.* Comparison of shear bond strength relative to two testing devices. J Prosthet Dent 2002; 88(5): 511−5.
18. *Placido E, Meira JB, Lima RG, Muench A, Souza RM, Ballester RY.* Shear versus micro-shear bond strength test: A finite element stress analysis. Dent Mater 2007; 23(9): 1086−92.
19. *McDonough WG, Antonucci JM, He J, Shimada Y, Chiang MY, Schumacher GE,* et al. A microshear test to measure bond strengths of dentin-polymer interfaces. Biomaterials 2002; 23(17): 3603−8.
20. *Van Meerbeek B, Peumans B, Poitevin M, Mine A, Van A, Ende A,* et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010; 26(2): e100−21.
21. *Tedesco TK, Garcia EJ, Maxnuck Soares FZ, de Oliveira Rocha R, Grande RH.* Effect of two microsheartest devices on bond strength and fracture pattern in primary teeth. Braz Dent J 2013; 24(6): 605−9.
22. *Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R*, et al. Relationship between surface area for adhesion and tensile bond strength: evaluation of a micro-tensile bond test. Dent Mater 1994; 10(4): 236−40.
23. *Phrukkanon S, Burrow MF, Tyas MJ.* The influence of cross-sectional shape and surface area on the microtensile bond test. Dent Mater 1998; 14(3): 212−21.
24. *Abreu CW, Santos JF, Passos SP, Michida SM, Takahashi FE, Bottino MA.* The influence of cutting speed and cutting initiation location in specimen preparation for the microtensile bond strength test. J Adhes Dent 2011; 13(3): 221−6.
25. *Raposo LH, Armstrong SR, Maia RR, Qian F, Geraldeli S, Soares CJ.* Effect of specimen gripping device, geometry and fixation method on microtensile bond strength, failure mode and stress distribution: laboratory and finite element analyses. Dent Mater 2012; 28(5): e50−62.
26. *Nujella BP, Choudary MT, Reddy SP, Kumar MK, Gopal T.* Comparison of shear bond strength of aesthetic restorative materials. Contemp Clin Dent 2012; 3(1): 22−6.
27. *Allanigue CM, Rapp R, Piesco NP, Elliott MA, Nirschl RF, Guevara PA,* et al. Adaptation of composite resin restorative materials to retentive grooves of Class I cavity preparations. Pediatr Dent 1986; 8(3): 147−52.
28. *Braz R, Sinhoreti MA, Spazzin AO, Loretto SC, Lyra AM, Meira-Júnior AD.* Shear bond strength test using different loading conditions: A finite element analysis. Braz J Oral Sci 2010; 9(4): 439−42.
29. *Hilton TJ.* Can modern restorative procedures and materials reliably seal cavities? In vitro investigations. Part 1. Am J Dent 2002; 15(3): 198−210.
30. *Schuurs AH, Gruythuysen RJ, Wesselink PR.* Pulp capping with adhesive resin-based composite vs. calcium hydroxide: A review. Endod Dent Traumat 2000; 16(6): 240−50.
31. *Stojanovska V, Stevanović M, Mitić A.* In vitro comparison of shear bond strength of two resin composites to enamel and dentine using Excite as an adhesive. Acta Stomatol Naissi 2005; 21(50): 429−36. (Serbian)
32. *Khatri A, Nandlal B, Srilatha M.* Comparative evaluation of shear bond strength of conventional composite resin and nanocomposite resin to sandblasted primary anterior stainless steel crown. J Indian Soc Pedod Prev Dent 2007; 25: 282−5.
33. *Dominguez MS, Witherspoon DE, Gutmann JL, Opperman LA.* Histological and Scanning Electron Microscopy Assessment of Various Vital Pulp-Therapy Materials. J Endod 2003; 29(5): 324−33.
34. *Bona AD, Pinzetta C, Rosa V.* Effect of acid etching of glass ionomer cement surface on the microleakage of sandwich restorations. J Oral Sci 2007; 15(3): 230−4.

35. *El-Askay FM, Nassif MS, Andrade AM, Reis A, Loguercio AD.* Effect of surface area and air drying distance on shear strength of etch-and-rinse adhesive. Braz Oral Res 2012; 26: 418−23.
36. *Khoroushi M, Rafiei E.* Effect of thermocycling and water storage on bond longevity of two self-etch adhesives. Gen Dent 2013; 61(3): 39−44.
37. *Burger KM, Cooley RL, Garcia-Godoy F.* Effect of thermocycling times on dentin bond strength. J Esthet Dent 1992; 4(6): 197−8.
38. *Helvatjoglu-Antoniades M, Koliniotou-Kubia E, Dionyssopoulos P.* The effect of thermal cycling on the bovine dentine shear bond strength of current adhesive systems. J Oral Rehabil 2004; 31(9): 911−7.
39. *Miyazaki M, Sato M, Onose H, Moore BK.* Influence of thermal cycling on dentin bond strength of two-step bonding systems. Am J Dent 1998; 11(3): 118−22.
40. *Siqueira JF, Rocas IN, Abad EC, Castro AJ, Gahyva SM, Favieri A.* Ability of three root-end filling materials to prevent bacteria leakage. J Endod 2001; 27(11): 673−5.
41. *Gaengler P, Hoyer I, Montag R.* Clinical evaluation of posterior composite restorations: the 10-year report. J Adhes Dent 2001; 3(2): 185−94.
42. *Davidson Cl, de Gee AJ, Fielzer A.* The competition between the composite-dentin bond strength and the polymerization contraction stress. J Dent Res 1983; 63(12): 1396−9.
43. *Munksgaard EC, Irie M, Asmussen E.* Dentin-polymer bond promoted by Gluma and various resins. J Dent Res 1985; 64(12): 1409−11.
44. *von Fraunhofer JA, Marshall KR, Holman BG.* The effect of base / liner use on restoration leakage. Gen Dent 2006; 54(2): 106−9.
45. *Murray PE, García-Godoy F.* The incidence of pulp healing defects with direct capping materials. Am J Dent 2006; 19(3): 171−7. 46.
46. *Dickens SH, Kelly SR, Flaim GM, Giuseppetti AA.* Dentin adhesion and microleakage of a resin-based calcium phosphate pulp capping and basing cement. Eur J Oral Sci 2004; 112(5): 452−7.
47. *Triana R, Prado C, Garro J, García-Godoy F.* Dentin bond strength of fluoride-releasing materials. Am J Dent 1994; 7(5): 252−4.
48. *Bell R, Barkmeier WW.* Glass-ionomer restoratives and liners: Shear bond strength to dentine. J Esthet Dent 1994; 6(3): 129−34.
49. *Fortin D, Vargas MA, Swift EJ.* Bonding of resin composites to resin-modified glass ionomers. Am J Dent 1995; 8(4): 201−4.
50. *Lin A, Mcintyre NS, Davidson RD.* Studies on adhesion of glass-ionomer cement to dentin. J Dent Res 1992; 71(11): 1936−41.
51. *Opdam NJ, Bronkhorst EM, Roeters JM, Loomans BA.* Longevity and reasons for failure of sandwich and total-etch posterior composite resin restorations. J Adhes Dent 2007; 9(5): 469−75.
52. *Goracci G, Mori G.* Scanning electron microscopic evaluation of resin-dentin and calcium hydroxide-dentin interface with resin composite restorations. Quintessence Int 1996; 27(2): 129−35.
53. *Estrela C, Holland R.* Calcium hydroxide: Study based on scientific evidences. J Appl Oral Sci 2003; 11(4): 269−82.
54. *Cheung G.* An in vitro evaluation of five dentinal adhesives in posterior restorations. Quintessence Int 1990; 21(6): 513−6.

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A quantitative analysis of two-dimensional manually segmented transrectal ultrasound axial images in planning high dose rate brachytherapy for prostate cancer

Kvantitativna analiza aksijalnih dvodimenzionalnih ručno segmentisanih slika dobijenih primenom transrektalnog ultrazvuka u planiranju brahiterapije tumora prostate visokim brzinama doze

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Abstract

Background/Aim. Prostate delineation, pre-planning and catheter implantation procedures, in high-dose rate brachytherapy (HDR-BT), are commonly based on the prostate manually segmented transrectal ultrasound (TRUS) images. The aim of this study was to quantitatively analyze the consistency of prostate capsule delineation, done by a single therapist, prior to each HDR-BT fraction and the changes in the shape of the prostate capsule during HDR-BT, using two dimensional (2D) TRUS axial image. **Methods.** A group of 16 patients were treated at the Medical System Belgrade Brachytherapy Department with definitive HDR-BT. The total applied median dose of 52 Gy was divided into four individual fractions, each fraction being delivered 2–3 weeks apart. Real time prostate axial visualization and the manual segmentation prior to each fraction were performed using B-K Medical ultrasound. Quantitative analyses, analysis of an area and shape were applied on 2D-TRUS axial images of the prostate. Area analyses were used to calculate the average value of the cross-sectional area of the prostate image. The parameters of the prostate shape, the fractal dimension and the circularity ratio of the prostate capsule contour were estimated at the maximum axial cross section of the prostate image. **Results.** The sample group consisted of four phases, each phase being performed prior to the first, second, third and fourth HDR-BT fraction, respectively. Statistical analysis showed that during HDR-BT fractions there were no significant differences in the average value of area, as well as in the maximum shape of prostate capsule. **Conclusions.** Quantitative analysis of TRUS axial prostate segmented images shows a successful capsule delineation in the series of manually segmented TRUS images, and the prostate maximum shape remaining unchanged during HDR-BT fractions.

Key words:
prostatic neoplasms; ultrasonography; brachytherapy; models, theoretical.

Apstrakt

Uvod/Cilj. Ocrtavanje kapsule prostate, preplaniranje i procedure primene katetera u brahiterapiji visokim brzinama doze [*high-dose rate brachytherapy* (HDR-BT)] zasniva se na ručnoj segmentaciji transrektalne ultrazvučne (TRUS) slike. Cilj rada bio je da se korišćenjem dvodimenzionalne TRUS aksijalne slike kvantitativno analizira konzistentnost ocrtavanja kapsule prostate za svaku HDR-BT frakciju, kao i promena oblika kapsule prostate tokom HDR-BT. **Metode.** Na grupu od 16 bolesnika bila je primenjena definisana HDR-BT u Odeljenju za brahiterapiju privatne opšte bolnice – Medicinski sistem Beograd. Ukupna primenjena srednja doza na metu od 52 Gy bila je podeljena u četiri individualne frakcije i svaka frakcija primenjena sa pauzom od dve do tri nedelje. Aksijalna vizualizacija prostate i ručno segmentisanje pre svake fracije izvedeno je pomoću ultrazvučnog uređaja B-K Medical. Ocrtavanje je vršio isti terapeut. Kvantitativna analiza, analiza površine i oblika prostate bila je primenjena na 2D-TRUS aksijalne slike prostate za svakog bolesnika posebno. Analiza površine koristila se za izračunavanje srednje vrednosti površine poprečnog preseka prostate. Parametri oblika prostate, fraktalna dimenzija i cirkularnost konture kapsule prostate bili su procenjeni na slici najvećeg poprečnog preseka prostate.

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Rezultati. Ispitivani uzorak obuhvatio je četiri faze (TRUS prostate pre prve, druge, treće i četvrte HDR-BT frakcije). Statistička analiza pokazala je da u toku HDR-BT frakcije nije bilo značajne razlike u srednjoj vrednosti površine, kao ni u obliku kapsule prostate na njenom najvećem preseku. **Zaključak.** Kvantitativna analiza TRUS aksijalne segmetisane slike prostate pokazala je uspešno ocrtavanje prostate u seriji ručno segmentisanih TRUS slika i nepromenjen oblik prostate na najvećem njenom preseku u toku HDR-BT frakcija.

Ključne reči:
prostata, neoplazme; ultrasonografija; brahiterapija; modeli, teorijski.

Introduction

Prostate carcinoma is a common cancer and the second most common cause of cancer deaths in males [1]. Therapeutic management of prostate carcinoma has become complex, multidisciplinary and stage-specific [2, 3]. Treatment options for patients with localized, organ confined prostate carcinoma range from observation, radical prostatectomy, radiation therapy (external and/or brachytherapy), hormonal therapy and various combinations of these [3–5].

Prostate brachytherapy (BT) is one of definitive treatment options for low risk organ confined carcinoma [6–8] and intermediate-risk prostate carcinoma [9–14]. BT dose patterns and fractionation predominantly depend on BT modality [low-dose rate (LDR) or high-dose rate (HDR)] [15–17], disease stage, tumor/target volume, therapy scope (definitive/monotherapy, boost or salvage), status of the organs at risk (urethra and rectum) and patient performance status.

In practice, the final prostate contours for BT pre-planning or BT post-implantation planning could be obtained using manual, semi-automatic or automatic segmentation of the prostate margin images obtained by computed tomography (CT), magnetic resonance image (MRI) or transrectal ultrasound (TRUS) [18–23]. However, the prostate delineation, BT pre-planning and catheter implantation BT procedures are commonly based on the use of manually segmented TRUS images obtained in axial and sagittal plains [6, 15, 22] (Figure 1).

Precise information about the size and the shape of prostate plays a key role in BT implantation and planning processes. Manual TRUS prostate capsule segmentation (delineation) is known to be difficult and hardly reproducible due to interference of numerous unfavorable factors [24, 25]. Despite these downsides, TRUS in the prostate diagnostics value is not inferior to CT or MRI [24].

An important issue in prostate BT is the quality and consistency of prostate segmentation by TRUS. It is well-known that human expert performance (urologist and/or radiation oncologist/brachytherapist) in manual segmentation, measured as the prostate BT workload, can affect final therapy result [26], especially when fractionated high dose rate BT (HDR-BT) treatment planning is based on TRUS segmented prostate images.

However, when prostate HDR-BT is concerned, more than one implantation procedure could be applied. In these cases, it would be beneficial to have data on quantitative analysis of precision and consistency in prostate two dimensional (2D) TRUS axial image segmentation. Also, it would be beneficial to have precise information about the shape of the prostate during BT. Therefore the purpose of this study was to analyze consistency of the prostate capsule delineation prior to each HDR-BT fraction and the changes in the axial shape of the prostate capsule during HDR-BT using TRUS imaging.

Methods

Patients

A group of 16 patients, aged 65–82 years, in good health condition and performance status were treated at the Medical System Belgrade Brachytherapy Department, with definitive temporary HDR-BT (Microselectron HDR, ELECTA-Nucletron, the Netherlands). The total applied median dose was 52 Gy divided into four individual fractions, each fraction being delivered 2–3 weeks apart. The total HDR-BT dose was tailored individually, based on the selected association criteria of the American Brachytherapy Society [27]. The applied HDR-BT technique has been explained in detail elsewhere [4, 28]. Androgen blockade therapy was applied to the group of patients 2–3 months prior to HDR-BT in order to intensify prostate encapsulation and shrink prostate volume (not necessarily uncirculated).

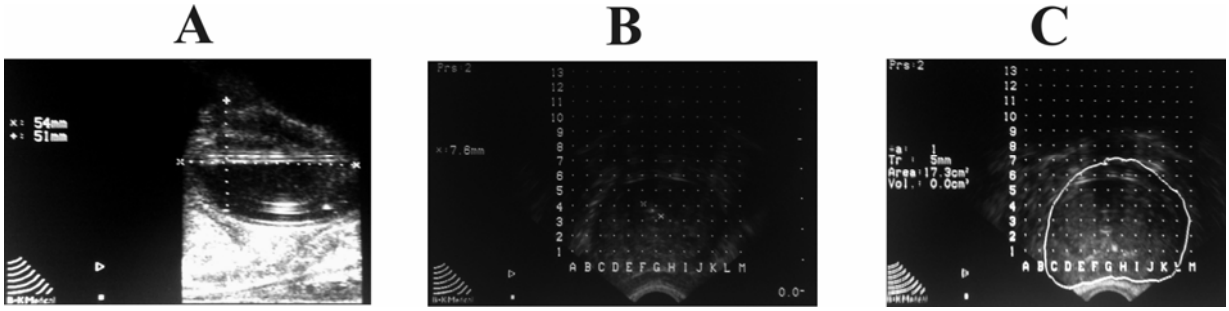

Fig. 1 – Typical transrectal ultrasound (TRUS) images of the prostate, shown with permission of the General Hospital Medical System Belgrade (MSB): A) sagittal plan, B) axial plan and C) segment axial plan at the maximum prostate cross-sectional area.

TRUS data acquisition

Patients were placed in lithotomy position under spinal anesthesia. Two parallel needles were positioned laterally to the prostatic urethra (Figure 1C) in order to prevent prostate movement during TRUS imaging and afterwards needle placement. Real time prostate axial visualization and manual segmentation prior each fraction was performed using B-K Medical Ultrasound (Model Hawk 2102) with a 7.5 MHz biplane TRUS probe mounted on a prostate-stepper. Manual segmentation, done by a single therapist, was performed from the prostate base (image b_0) to the prostate apex (b_a), using a $5 \cdot 10^{-3}$ m step (0 denotes a segment position from the prostate base and apex segment position). Prior to segmentation, Nucletron $5 \cdot 10^{-3}$ m grid was attached, centered and calibrated, allowing accurate rigid catheter/needle insertion. The median prostate volume calculated from TRUS data (V_{pc}) and volume (V_p) obtained by other means (CT or MRI), were $56 \cdot 10^{-6}$ m^3 (range $30–85 \cdot 10^{-6}$ m^3), and 43 cm^3 (range $30–68 \cdot 10^{-6}$ m^3), respectively, with the mean V_{pc}/V_p ratio of 1.4. The prostatic urethra volume was included in the calculated prostate volume.

For each patient, four imaging phases were completed and recorded: phase I – segmentation data collected before the first HDR-BT fraction; phase II – segmentation data collected before the second HDR-BT fraction; phase III – segmentation data collected before the third HDR-BT fraction; phase IV – segmentation data collected before the fourth HDR-BT fraction. A representative TRUS images prostate segment at the maximum axial cross sections (Figure 2) was used for HDR-BT pre-planning and catheter/needle application. The difference of maximum axial cross sections shown in Figure 2 can be directly related to the prostate volume changes between HDR-BT fractions. Post-therapy TRUS in the treated patients was not scheduled since this type of examination was not mandatory in HDR post-treatment evaluation protocol.

Image processing

All axial 2D-TRUS images were imported into the Image J, specialized public domain software for image analysis, developed by the National Institute of Health (USA, www.rsb.info.nih.gov/ij). Schematic representation of TRUS image processing procedure is illustrated in Figure 3. In the first step of the procedure grey-scale (Figure 3B) images were converted into the binary images (Figure 3A) using 'Threshold' tools. Figure 3B shows the silhouette area of the prostate capsule. In the second step of the procedure, binary images (Figure 3B) were converted into outline images (Figure 3B) using 'Outline' tools. Figure 3C shows the contour of the prostate capsule.

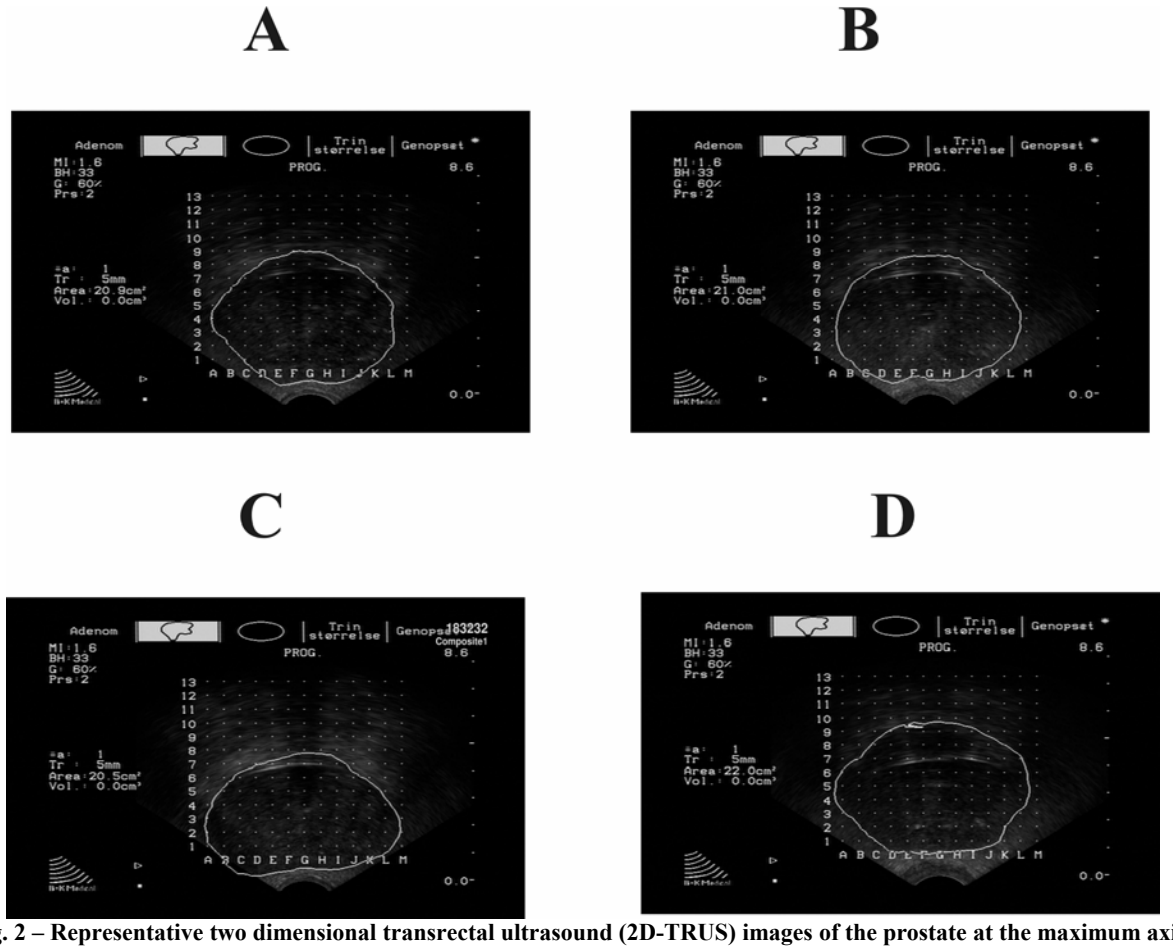

Fig. 2 – Representative two dimensional transrectal ultrasound (2D-TRUS) images of the prostate at the maximum axial cross section for the individual patient: A) phase I; B) phase II; C) phase III; D) phase IV.

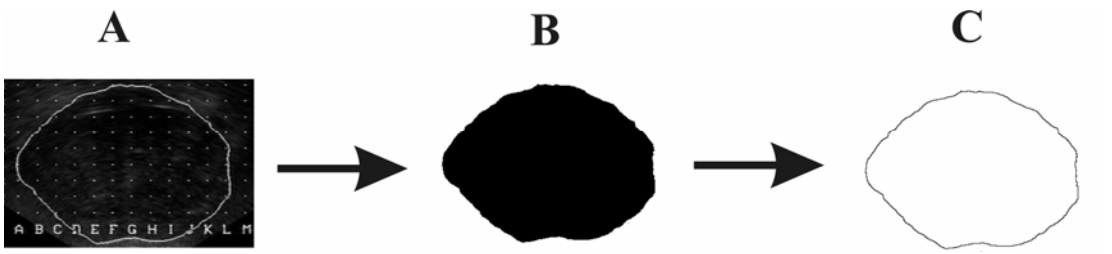

Fig. 3 – Schematic representation of the two-dimensional transrectal ultrasound (2D-TRUS) axial image of the prostate capsule processing procedure: grayscale image (A), image of the silhouette area (B) and of contour (C).

Quantitatively analyses of two dimensional TRUS axial image of the prostate capsule

Area analysis of the TRUS axial image of the prostate capsule

The cross sectional area of the prostate capsule (A) was measured on a particular 2D-TRUS axial image. The area measurement was carried out using TRUS standard calculation algorithm by B-K Medical Ultrasound (Model Hawk 2102). The average value of the cross-sectional area of the prostate (A_{av}) for segmentation data collected before the first, second, third and the fourth HDR-BT fraction (phase I; phase II; phase III; phase IV) was calculated (Figure 4).

Shape analysis of the TRUS axial image of the prostate capsule

Shape analysis of the prostate was estimated by two parameters: the fractal dimension and the circularity ratio of the prostate capsule contour. The parameters of the prostate shape were estimated at the maximum cross section. These parameters were obtained by using Image J.

Fractal dimension of the prostate capsule contour

The fractal dimension of the prostate contour at the maximum axial cross-section (D_{max}) defines the irregularity in the shape of the image, showing shape deviation from the corresponding circle (D_{circle} = 1.254) (Figure 5A). The image of the contour was analyzed by fractal analysis using the box-counting method [29, 30]. This method consists of "covering" the image outline with sets of squares (Figure 5B). Each set is characterized by the size r of the square edge. The corresponding number of squares N that is necessary to cover the image outline is presented as a function of r (Figure 5C). D_{max} is determined from the absolute slope value of the log–log relationship between $N(r)$ and r (Figure 5C). In performing box-counting method, the box sizes were scaled to the base of 2; that is, 2^1, 2^2... 2^k, where k continues until N is equal to unity [31]. Depending on the contour image size, the box-sizes were taken from 2 to 512 pixels.

Circularity of the contour prostate capsule

Although the prostate contours seem generally circular by nature, in practice we noticed a significant deviation of the prostate contours from the circle. A shape of the prostate capsule contour at the maximum axial cross section was estimated by using the circularity ratio (C_{max}). This parameter represents a measure of how particular shape deviates from a corresponding circle. If it is a circle contour, the circularity ratio is equal to one. In case of any other contour shape, the circularity ratio is less than one. This parameter is expressed as $C = 4\pi AP^{-2}$, where A is the area and P is the perimeter of the contour (Figure 6) [31].

Statistical analysis

Statistical analysis of the calculated prostate parameters depends on whether the distribution is normal or not. In the

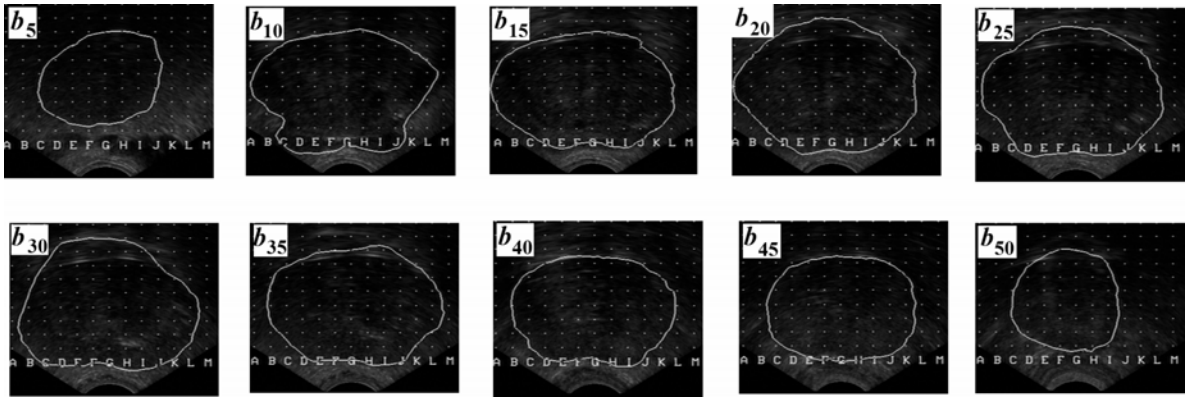

Fig. 4 – Two-dimensional transrectal ultrasound (2D-TRUS) axial images of prostate, from the prostate base (b_0) to the prostate apex (b_{50}). The cross-sectional area of the prostate capsule (A) from phase I brachytherapy in one patient; b_5 (A = $9.9\cdot10^{-4}$ m^2), b_{10} (A = $10.5\cdot10^{-4}$ m^2), b_{15} (A = $19.8\cdot10^{-4}$ m^2), b_{20} (A = $20.9\cdot10^{-4}$ m^2), b_{25} (A = $20.1\cdot10^{-4}$ m^2), b_{30} (A = $20.3\cdot10^{-4}$ m^2), b_{35} (A = $18.3\cdot10^{-4}$ m^2), b_{40} (A = $15.6\cdot10^{-4}$ m^2), b_{45} (A = $14.5\cdot10^{-4}$ m^2), b_{50} (A = $10.1\cdot10^{-4}$ m^2).

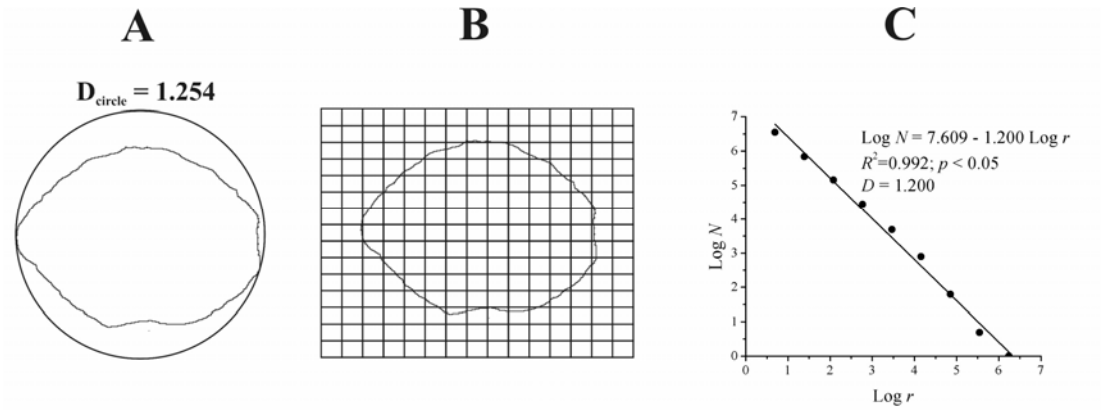

Fig. 5 – Illustration of calculating the fractal dimension of the prostate capsule contour, the fractal dimension of the circle is inscribed in the upper part of the figure (A). The application of the box-counting method to the contour, the whole image is covered with a set of squares and the squares which cover inner contour are counted (B). Log–log plot between the numbers of squares (*N*) and square size (*r*) is fitted by a straight line (C). The fractal dimension is 1.200, R^2 is the corresponding determination coefficient and *p* is the significance level.

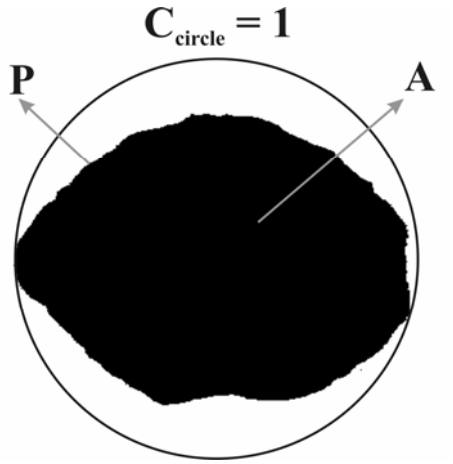

Fig. 6 – Illustration of circularity ratio calculation for the prostate capsule contour ($C = 4\pi AP^2$, *A* is the area and *P* is the perimeter). The circularity ratio of the circle is inscribed in the upper part of the figure.

case of calculated parameters showing a normal distribution, each group is described by the corresponding mean value and standard error [32]. If the number of patients is smaller than 30, the character of distribution has to be tested by calculation of two statistical parameters: skewness, a_3 and excess of distribution, e [33]. Briefly, the intervals of skewness and excess values, which define the normal distribution, are estimated when these two parameters are divided by the corresponding mean square errors (σ_3 and σ_4). If the absolute value of the quotients σ_3/a_3 and σ_4/e is less than or equal to 2, the data distribution can be considered as normal. Otherwise, the distribution of a sample (or population) has no characteristics of a normal distribution [34]. A statistical significance between mean values of parameters of prostate for 4-phase brachytherapy was estimated by Student´s *t*-test [34].

Results

The sample group consisted of four phases, each phase being performed prior to the first (phase I), second (phase II), third (phase III) and the fourth (phase IV) BT fraction, respectively. Their representative axial images are shown in Figure 2. Table 1 shows value of the absolute ratios σ_3/a_3 and σ_4/e, for the average value of area (A_{av}), the fractal dimension (D_{max}) and the circularity ratio (C_{max}) for all four phases,. The absolute ratios (σ_3/a_3) and (σ_4/e) for the two parameters in all four of the phases are smaller than the critical value of 2. Therefore, the calculated values of three parameters showed normal distribution. Consequently, the values of three parameters of prostate capsule (A_{av}, D_{max} and C_{max}) can be expressed by the mean value and standards error.

The mean and standard errors of the A_{av}, D_{max} and C_{max} for four phases were presented in Table 2. It was noticed that all phases had similar mean values of the three parameters of the prostate capsule. The statistical test estimated the difference between the parameters of mean values for 4-phase brachytherapy.

In the next step, the mean values of all possible pairs were tested by *t*-test (Table 3). The *t*-values of all three parameters are less than the *t*-values tabulated at the level of

Table 1

Values of σ_3/a_3 and σ_4/e for the average value of the area (A_{av}), the fractal dimension (D_{max}) and the circularity ratio (C_{max}) of the prostate capsule for 4-phase high-dose rate brachytherapy (HDR-BT)

Phase	Parameter of the prostate capsule					
	A_{av}		D_{max}		C_{max}	
	σ_3/a_3[e]	σ_4/e[f]	σ_3/a_3[e]	σ_4/e[f]	σ_3/a_3[e]	σ_4/e[f]
I[a]	0.28	0.78	0.13	0.22	0.27	0.67
II[b]	0.22	0.51	0.27	0.79	0.33	0.95
III[c]	0.28	0.89	0.13	0.22	0.14	0.22
IV[d]	0.30	1.07	0.33	0.99	1.72	0.92

[a]Segmentation data collected before the first HDR-BT fraction; [b]segmentation data collected before the second HDR-BT fraction; [c] segmentation data collected before the third HDR-BT fraction; [d]segmentation data collected before the fourth HDR-BT fraction; [e]mean square errors/skewness values; [f]mean square errors/excess values.

Table 2

The average value* of the area (A_{av}), the fractal dimension (D_{max}), and the circularity (C_{max}) of the prostate capsule for 4-phases high-dose rate brachytherapy (HDR-BT).

Phase	$A_{av} \cdot 10^4$ (m^2)	D_{max}	C_{max}
I[a]	13 ± 3	1.19 ± 0.04	0.75 ± 0.07
II[b]	13 ± 2	1.19 ± 0.04	0.74 ± 0.06
III[c]	12 ± 3	1.19 ± 0.03	0.76 ± 0.03
IV[d]	13 ± 3	1.19 ± 0.04	0.75 ± 0.04

For abbreviations see under Table 1

***Each value is presented as the mean ± standard error.**

Table 3

The results of Student's *t*-test: *t*-values of the three parameters (A_{av}, D_{max} and C_{max}) of the prostate capsule given in Table 2 over pairs of the phases of high-dose rate brachytherapy (HDR-BT), tested for the significance

Pair of phases*	A_{av}	D_{max}	C_{max}
I[a]-II[b]	0.453	0.317	0.412
I[a]-III[c]	1.102	0.127	0.413
I[a]-IV[d]	0.605	0.102	0.437
II[b]-III[c]	0.766	0.226	1.060
II[b]-IV[d]	0.229	0.233	0.039
III[c]-IV[d]	0.456	0.021	1.389
$t_{0.05}$	2.131	2.131	2.131
$t_{0.01}$	2.947	2.947	2.947

For abbreviations see under Table 1

significance $p < 0.05$ and $p < 0.01$ (Table 3). The *t*-test showed that there were no statistical differences between all possible pairs of the means A_{av}, D_{max} and C_{max}, respectively (Table 3). Table 3 shows no significant difference in the values of A_{av}, D_{max} and C_{max}, for phase I, phase II, phase III and phase IV, respectively.

Discussion

TRUS is used to guide placement of implants during prostate BT [21], and as an input data for HDR-BT planning. This study quantitatively analyzed 2D-TRUS axial image obtained during prostate HDR-BT fractions. The average value area of cross section (A_{av}) and the two parameters relative to the maximum prostate capsule shape (D_{max} and C_{max}) were determined by analyzing 2D-TRUS axial image. Statistical analysis showed no significant differences in the average value of cross sectionsal area during, HDR-BT fractions (phase I, phase II, phase III and phase IV) as well as in the maximum shape of prostate capsule (D_{max} and C_{max}) (Table 3).

The absence of a significant difference in the average value of area (A_{av}) (Table 3) during prostate capsule delineation in the series of manually segmented TRUS images (phase I, phase II, phase III and phase IV) points to the consistency in prostate capsule delineation. This is in agreement with Solhjem et al. [19], who demonstrated a significant reproducibility in prostate volume measurement using TRUS. However, manual TRUS prostate capsule delineation is known to be hardly reproducible due to the interference of numerous unfavorable factors [24, 25]. This is the first time that the consistency in capsule delineation using TRUS was quantitatively proved, thus confirming a reproducibility of the manual TRUS segmented prostate axial images in preplanning and catheter/needle application for the HDR-BT.

On the other hand, our results show that the maximum prostate shape (D_{max} and C_{max}) remains unchanged during analysis of HDR-BT fractions (Table 3). Holmes et al. [21] conducted shape analysis of the prostate, however, the fractal dimension and the circularity were used for the first time in our study to quantify the shape of the prostate capsule. The fractal dimension is a geometrical feature of irregularly shaped

objects [35]. Fractal analysis was applied to different medical images [36], but there is no data on its application to the 2D-TRUS axial images of the prostate. Also, it is analyzed by means of the circularity, now commonly used as a parameter which quantifies the shape of two-dimensional objects [31].

The lack of a significant difference in the shape of axial TRUS images during prostate capsule delineation in the series of manually segmented TRUS images (phase I, phase II, phase III and phase IV), could point to the absence of geometrical change in the fractions of HDR-BT. However, post HDR-BT data was not collected as TRUS in the treated patients. It was not mandatory in the HDR post-treatment evaluation protocol. Consequently, data for analysis of the prostate shape after the last treatment was not available.

The parameters of the cross-sectional prostate capsule (A_{av}, D_{max} and C_{max}) show that manually segmented TRUS images display good visibility necessary for precise delineation and monitoring of the prostate behavior during HDR-BT.

It was documented that prostate gland shrinkage may be expected in patients who undergo external beam radiotherapy shortly after starting androgen deprivation therapy [37]. Considering external beam radiotherapy alone, a particular effect of prostate gland shrinkage seems to be clinically insignificant [38].

Conclusion

In this study, the applied quantitative analysis of transrectal ultrasound axial prostate segmented images obtained in each phase shows a successful capsule delineation in the series of manually segmented transrectal ultrasound images done by a single therapist and that the prostate maximum axial shape remains unchanged during the high-dose rate brachytherapy course, of approximately 2-month duration.

This is the first time to determine the prostate capsule maximum shape (the fractal dimension and the circularity) of transrectal ultrasound axial images. These two prostate shape parameters may be applicable in quantification of prostate shape for the purpose of medical diagnostics and treatment. Transrectal ultrasound of the prostate is fast and relatively inexpensive diagnostic technique. Quantitative analysis showed that transrectal ultrasound of the prostate is accurate in pre-planning and catheter/needle application to high-dose rate brachytherapy. Additionally, the applied image analysis could be suitable for determination of the accuracy in other radiotherapy planning techniques.

REFERENCES

1. *Parkin DM, Pisani P, Ferlay J.* Global cancer statistics. CA Cancer J Clin 1999; 49(1): 33−64, 1.
2. *Acimovic M, Dabic-Stankovic K, Pejcic T, Dzamic Z, Rafailovic D, Hadzi-Djokic J.* Preoperative Gleason score, percent of positive prostate biopsies and PSA in predicting biochemical recurrence after radical prostatectomy. J BUON 2013; 18(4): 954−60.
3. *Heidenreich A, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V*, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol 2011; 59(1): 61−71.
4. *Arsenijević T, Dabić-Stanković K, Aćimović M, Radošević-Jelić LJ.* Radiotherapy in Prostate Cancer.In: *Spiess* P, editor. Prostate Cancer: Diagnostic and Therapeutic Advances. Rijeka, Croatia: InTech; 2011.
5. *Mitsuzuka K, Koie T, Narita S, Kaiho Y, Yoneyama T, Tsuchiya N*, et al. Pathological and oncological outcomes of elderly men with clinically localized prostate cancer. Jpn J Clin Oncol 2013; 43(12): 1238−42.
6. *Blasko J, Radge H, Schumacher D.* Transperineal percutaneous iodine-125 implantation for prostatic carcinoma using tranrectal ultrasound and temlate guidance. Endocuriether Hypertherm Oncol 1987; 3: 131–9.
7. *Blasko JC, Wallner K, Grimm PD, Ragde H.* Prostate specific antigen based disease control following ultrasound guided 125iodine implantation for stage T1/T2 prostatic carcinoma. J Urol 1995; 154(3): 1096−9.
8. *Blasko JC, Grimm PD, Sylvester JE, Badiozamani KR, Hoak D, Cavanagh W.* Palladium-103 brachytherapy for prostate carcinoma. Int J Radiat Oncol Biol Phys 2000; 46(4): 839−50.
9. *Barkati M, Williams SG, Foroudi F, Tai KH, Chander S, van Dyk S,* et al. High-dose-rate brachytherapy as a monotherapy for favorable-risk prostate cancer: a Phase II trial. Int J Radiat Oncol Biol Phys 2012; 82(5): 1889−96.
10. *Demanes DJ, Martinez AA, Ghilezan M, Hill DR, Schour L, Brandt D*, et al. High-dose-rate monotherapy: safe and effective brachytherapy for patients with localized prostate cancer. Int J Radiat Oncol Biol Phys 2011; 81(5): 1286−92.
11. *Grills IS, Martinez AA, Hollander M, Huang R, Goldman K, Chen PY,* et al. High dose rate brachytherapy as prostate cancer monotherapy reduces toxicity compared to low dose rate palladium seeds. J Urol 2004; 171(3): 1098−104.
12. *Hoskin P, Rojas A, Lowe G, Bryant L, Ostler P, Hughes R,* et al. High-dose-rate brachytherapy alone for localized prostate cancer in patients at moderate or high risk of biochemical recurrence. Int J Radiat Oncol Biol Phys 2012; 82(4): 1376−84.
13. *Rogers CL, Alder SC, Rogers RL, Hopkins SA, Platt ML, Childs LC,* et al. High dose brachytherapy as monotherapy for intermediate risk prostate cancer. J Urol 2012; 187(1): 109−16.
14. *Yoshioka Y, Konishi K, Sumida I, Takahashi Y, Isohashi F, Ogata T,* et al. Monotherapeutic high-dose-rate brachytherapy for prostate cancer: five-year results of an extreme hypofractionation regimen with 54 Gy in nine fractions. Int J Radiat Oncol Biol Phys 2011; 80(2): 469−75.
15. *Das KR.* Prostate brachytherapy: HDR or seed implant. J Med Phys 2006; 31(4): 239−41.
16. *Cosset JM, Haie-Meder C.* Brachytherapy for prostate cancer: high dose rate or low-dose rate? Cancer Radiother 2005; 9(8): 610−9. (French)
17. *King CR.* LDR vs. HDR brachytherapy for localized prostate cancer: The view from radiobiological models. Brachytherapy 2002; 1(4): 219−26.
18. *Kälkner KM, Kubicek G, Nilsson J, Lundell M, Levitt S, Nilsson S,* et al. Prostate volume determination: Differential volume measurements comparing CT and TRUS. Radiother Oncol 2006; 81(2): 179−83.
19. *Sollhjem MC, Davis BJ, Pisansky TM, Wilson TM, Mynderse LA, Herman MG*, et al. Prostate volume measurement by transrectal ultrasound and computed tomography before and after per-

manent prostate brachytherapy. Int J Radiat Oncol Biol Phys 2004; 60(3): 767−76.
20. *Buch K, Morancy T, Kaplan I, Qureshi MM, Hirsch AE, Rofksy NM*, et al. Improved dosimetry in prostate brachytherapy using high resolution contrast enhanced magnetic resonance imaging: a feasibility study. J Contemp Brachytherapy 2015; 6(4): 337−43.
21. *Holmes DR 3rd, Davis BJ, Goulet CC, Wilson TM, Mynderse LA, Furutani KM,* et al. Shape analysis of the prostate: establishing imaging specifications for the design of a transurethral imaging device for prostate brachytherapy guidance. Brachytherapy 2014; 13(5): 465−70.
22. *Litjens G, Toth R, van de Ven W, Hoeks C, Kerkstra S, Ginneken B,* et al. Evaluation of prostate segmentation algorithms for MRI: The PROMISE12 challenge. Med Image Anal 2014; 18(2): 359−73.
23. *Mahdavi SS, Chng N, Spadinger I, Morris WJ, Salcudean SE*. Semi-automatic segmentation for prostate interventions. Med Image Anal 2011; 15(2): 226−37.
24. *Pfengfei W, Yiguang L, Yongzhong L, Liping C*. TRUS Image segmentation driwen by narrow band contrast pattern using shape space embedded level sets. In: *Jian Y, Fang F, Changyin S,* editors. Intelligent Science and Intelligent Data Engineering: Lecture Notes in Computer Science. Berlin, Heidelberg: Springer-Verlag; 2013. p. 339−46.
25. *Pingkun Y, Sheng X, Baris T, Kruecker J*. Discrete deformable model guided by partial active shape model for TRUS image segmentation. IEEE T Biomed Eng 2010; 57(5): 1158−66.
26. Thompson S, Delaney G, Gabriel G, Izard MA, Hruby G, Jagavkar R, et al. Prostate brachytherapy in New South Wales: Patterns of care study and impact of caseload on treatment quality. J Contemp Brachytherapy 2015; 6(4): 344−9.
27. *Yamada Y, Rogers L, Demanes DJ, Morton G, Prestidge BR, Pouliot J,* et al. American Brachytherapy Society consensus guidelines for high-dose-rate prostate brachytherapy. Brachytherapy 2012; 11(1): 20−32.
28. *Skowronek J*. Low-dose-rate or high-dose-rate brachytherapy in treatment of prostate cancer - between options. J Contemp Brachytherapy 2013; 5(1): 33−41.
29. *Milosević NT, Ristanović D, Marić DL, Rajković K*. Morphology and cell classification of large neurons in the adult human dentate nucleus: A quantitative study. Neurosci Lett 2010; 468(1): 59−63.
30. *Ristanović D, Milosević NT, Stefanović BD, Marić DL, Rajković K*. Morphology and classification of large neurons in the adult human dentate nucleus: A qualitative and quantitative analysis of 2D images. Neurosci Res 2010; 67(1): 1−7.
31. *Milosević NT, Ristanović D, Jelinek HF, Rajković K*. Quantitative analysis of dendritic morphology of the α and δ retinal ganglion cells in the rat: A cell classification study. J Theor Biol 2009; 259(1): 142−50.
32. *Alder H, Roessler EB*. Introduction to Probability and Statistics. San Francisco, USA: WH Freeman and Co; 1972.
33. *Rajković K, Bačić G, Ristanović D, Milošević NT*. Mathematical Model of Neuronal Morphology: Prenatal Development of the Human Dentate Nucleus. Biomed Res Int 2014; 2014: 1−8.
34. *Riffenburgh RH*. Statistics in Medicine. London, UK: Academic Press; 1999.
35. *Di Ieva A, Grizzi F, Sherif C, Matula C, Tschabitscher M*. Angioarchitectural heterogeneity in human glioblastoma multiforme: A fractal-based histopathological assessment. Microvasc Res 2011; 81(2): 222−30.
36. *Lopes R, Betrouni N*. Fractal and multifractal analysis: A review. Med Image Anal 2009; 13(4): 634−49.
37. *Sanguineti G, Marcenaro M, Franzone P, Foppiano F, Vitale V*. Neoadjuvant androgen deprivation and prostate gland shrinkage during conformal radiotherapy. Radiother Oncol 2003; 66(2): 151−7.
38. *Budiharto T, Slagmolen P, Hermans J, Maes F, Verstraete J, Heuvel FV,* et al. A semi-automated 2D/3D marker-based registration algorithm modelling prostate shrinkage during radiotherapy for prostate cancer. Radiother Oncol 2009; 90(3): 331−6.

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Effects of combined special education treatment and occupational therapy on upper extremities motor skills in adult patients with hemiplegia

Efekti kombinovanog somatopedskog lečenja i radne terapije na motoričke sposobnosti gornjih ekstremiteta odraslih bolesnika sa hemiplegijom

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Abstract

Background/Aim. Stroke is the most common single cause of severe and multiple physical disabilities, and rehabilitation that reduces functional deficits is the most effective treatment. The aim of this study was to determine the effect of special education treatment as a supplement to occupational therapy on upper extremities motor skills in adult patients with post stroke hemiplegia. **Methods.** Standard education tests for motor function evaluation of the upper extremities: O`Connor, Ring and Hand grip test, were applied on a sample of 64 patients who were in the process of rehabilitation in the Clinic for Rehabilitation "Dr. Miroslav Zotović" in Belgrade. After the evaluation, all the participants were included in occupational therapy and divided in two intervention groups *per* 32 subjects each. The patients from the first experimental group received individually dosed special education treatment which was performed for at least 12 weeks as a supplement together with occupational therapy, while patients from the second experimental group were only in the process of occupational therapy without special education treatment. At the end of the study the same tests were used to re-evaluate the level of motor abilities of the patients in both groups. **Results.**The patients from the first experimental group with individually dosed special education treatment as a supplement showed significantly better scores after applying the treatment in all tested variables – explosive, static and dynamic muscular strength grip fist, as well as oculomotor skills at the level of the elbow and shoulder for both healthy and paretic hand. **Conclusion.** On the basis of the obtained results, it can be concluded that special education treatment added to occupational therapy lead to better performing of upper extremities motor skills and that it can be a good supplement to conventional occupational therapy methods and techniques.

Key words:
hemiplegia; neurological rehabilitation; education, special; occupational therapy; upper extremities; adult.

Apstrakt

Uvod/Cilj. Moždani udar je najčešći pojedinačni uzrok teškog i višestrukog fizičkog invaliditeta, a rehabilitacija koja smanjuje funkcionalne deficite je najefikasnije lečenje. Cilj istraživanja bio je da se utvrdi uticaj programa specijalne obuke dodate radnoj terapiji za motoričke sposobnosti gornjih ekstremiteta kod odraslih bolelsnika sa hemiplegijom nastalom posle moždanog udara. **Metode.** Na uzorku od 64 bolesnika, koji su bili u procesu rehabilitacije u Klinici za rehabilitaciju „Dr Miroslav Zotović" u Beogradu, primenjeni su standardni somatopedski testovi za procenu motorike gornjih ekstremiteta: O`Connor, Ring i test snage stiska pesnice. Nakon procene, svi učesnici bili su uključeni u radnu terapiju i podeljeni u dve grupe po 32 ispitanika. Bolesnicima prve eksperimentalne grupe kao dopuna radnoj terapiji dodato je individualno dozirano somatopedsko lečenje u trajanju od najmanje 12 nedelja, dok su bolesnici iz druge eksperimentalne grupe bili uključeni samo u proces radne terapije bez somatopedskog lečenja. Na kraju istraživanja, isti testovi su korišćeni za ponovnu procenu nivoa motoričkih sposobnosti bolesnika iz obe grupe. **Rezultati.** Bolesnici iz eksperimentalne grupe koja je uz redovnu terapiju lečena i somatopedski pokazali su znatno bolje rezultate nakon primenjenog lečenja u svim ispitivanim varijablama – eksplozivna, statička i dinamička mišićna snaga stiska pesnice, kao i okulomotorna koordinacija na nivou lakta i ramena, kako za zdrav, tako i za paretičan ekstremitet. **Zaključak**. Na osnovu dobijenih rezultata, može se zaključiti da somatopedsko lečenje primenjeno uz radnu terapiju dovodi do poboljšanja motoričkih sposobnosti gornjih ekstremiteta i može biti dobar dodatak konvencionalnim metodama i tehnikama radne terapije.

Ključne reči:
hemiplegija; rehabilitacija, neurološka; edukacija, specijalna; radna terapija; ekstremiteti, gornji; odrasle osobe.

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Introduction

Acute stroke is defined as a focal or global disruption of brain function that occurs suddenly as the result of a cerebrovascular disorder or condition in which blood flow is not sufficient to meet the metabolic needs of the neurons for oxygen and glucose. The incidence of ischemic stroke varies and ranges from 100 to 300 new cases *per* year *per* 100,000 inhabitants, while the prevalence rate ranges from around 600 *per* 100,000 populations in developed countries, up to 900 in underdeveloped. Stroke is also the fifth leading cause of disability internationally [1–3]. Despite advances in medicine and initiatives to improve public health, the incidence of stroke is not reduced [4, 5]. According to data from the Canadian Institute for Health Information, patients who survive stroke are the largest category of users of physical rehabilitation services and the third by the length of rehabilitation (after patients with spinal cord injuries and brain dysfunction [6]. The consequences of stroke are often serious and severe, and in addition to affecting the full range of human life and functioning they also have devastating effects on family members [7], bringing the experience of stress, depression, social isolation, threatening health and rarely leading to premature death. In spite of all the researchers efforts it is not yet established the one and only, single "therapeutic cure" for motor impairments of a hand in adults with post-stroke hemiplegia. Some excellent studies in which references to the operation of both conventional (neurodevelopment therapy or "Bobath method", functional electrical stimulation, biofeedback electromyographically etc.), as well as a newer, less traditional approach (Reiki method, robotics, etc.), reported that despite the considerable efforts of researchers aimed at proving that some treatment "works", no rehabilitation intervention is not allocated and proved to be a single "cure" for recovery after stroke [8–11]. After stroke, patients must be involved in rehabilitation in the first six months, when the largest neurological and functional recovery mostly manifest. Motor impairment is the most common deficit after stroke and the major contributor to functional limitations [12]. More than 80% of individuals with stroke experience hemiparesis and of those people who initially have upper-extremity paresis, it is estimated that 70% have residual impairment. The upper limb makes a significant contribution to most activities of daily living (ADL), and impairments can compromise participation in many of these essential and meaningful tasks 13. According to another research 14, during one-year after stroke, 52% of all patients improve functional walking ability and in only 15% hand function fully recovers. It is also noted that one year after a stroke, patients with limited upper extremity function have significantly poorer image of themselves and their own recovery. Upper-limb function return has been identified as an important rehabilitation goal 12. Occupational therapists use several methods of treatment and the choice of approach depends largely on the therapist's experience and preferences. Activities are oriented towards the process of re-education and fostering the development of lost skills, while the patient is adjusting to life with certain physical, cognitive, and affective disorders. Occupational therapy treatment is aimed at preventing the development of contractures, normalization of postural tone, inhibiting abnormal patterns of posture and movement and facilitating normal postural patterns of standing upright, balance and adaptive changes in muscle tone [15, 16]. Another set of goals in occupational therapy is to achieve and establish active and voluntary control of trunk and extremities movements, volitional control of the affected hand when performing movements with the healthy arm, to establish control through shoulder and elbow flexion and extension synergy and voluntary control of rough primitive movements of the hand and fingers in capturing and releasing objects, and to combine simple movement patterns in performing complex activities of self-care together with performing daily activities using paretic hand. For patients with the plegic hand practicing compensatory function, primarily single-handed performance of bimanual training activities and practice the healthy hand to hyper function is the major task in occupational therapy [17, 18]. One approach in the treatment is teaching patients to do activities which are typically performed bimanual by with one hand. Another strategy of compensation is making changes of environment in order to reduce negative effects on the desired function of the injured limb. With the method of compensation and/or adaptation it is necessary in parallel to work on improvement of functional ability of weaker extremities [18, 19].

The aim of this study was to determine if there are any effects of combined special education treatment added to occupational therapy as a supplement to upper extremities motor skills in patients with hemiplegia who are in the process of occupational therapy.

Methods

The research was conducted during 2014/15 in occupational therapy units at the Clinic for Rehabilitation "Dr. Miroslav Zotović" in Belgrade. The research was approved by the Board of Ethical Committee of the Clinic (No.03-1908/1). All the participants were informed about the purpose of the study and signed the permission. The research was conducted in a clinical setting with the written agreement of all the included patients.

The study was conducted as a randomized controlled trial (RCT) or randomized controlled clinical trial (RCCT) – design that randomly assigns participants into experimental groups. In the RCT, an intervention is investigated by comparing one group of people who receive the intervention with the other group who does not, and the only expected difference between the groups in a randomized controlled trial is the outcome variable being studied. In this investigation both intervention groups were treated identically and received the usual occupational therapy treatment according to the protocol of the institution, but one group received special education treatment as a supplement to occupational therapy. Research conducted in this way can give an answer to practical question of whether introducing the new treatment (special education) could improve outcomes over and above the current state of practice.

Participants were chosen randomly from the groups of fully and partially hospitalized patients. The sample of patients with multiple strokes included those who were several times hospitalized at the Clinic, thus they were in a well-known environment, but there were also first time hospitalized patients. During the research from the first randomly chosen number of subjects, 8 patients were excluded because they finished rehabilitation and were discharged from the hospital and 6 subjects because they were not regular in visiting occupational therapy unit, so the final sample for the study comprised a total of 64 patients separated into two groups, 32 subjects each. Before the application of treatment both groups were equalized according to age, sex, the diagnosis and the level of motor skills. In both groups there were the equal number of men (18) and women (14), and the average age of the participants in the group who received only occupational therapy was slightly higher (63.61), compared to the group of patients who received special education exercises where the average age was 59.34 years. All the participants from both groups were prescribed occupational therapy according to the protocol of the clinic, and were included in it regularly. As a supplement to standard occupational therapy procedures, the patients from the first intervention group received a set of personalized exercises from the special education program while the patients from the second intervention group had only occupational therapy without special education exercises as a supplement.

In order to achieve the set goals first it was necessary to establish a personal card for each subject to record age, gender and the medical diagnosis. This general data were obtained from the available medical documentation. Data for dependent variables identifying the degree of motor impairment were collected by using both standard and special education evaluation methods and personal insight through appropriate diagnostic procedures. During the study the following dependent variables were examined: explosive muscular strength grip fist, static muscular strength grip fist, and dynamic muscular strength grip fist, oculomotor skills at the level of the elbow joint and oculomotor skills at the level of the shoulder. Each test was performed with both hands. Hand grip test is the test that measures explosive, static and dynamic muscular strength grip fist. Testing device consists of a pressure gauge and a rubber cuff (an apparatus for measuring blood pressure). Cuff inflates to 50 mmHg. On the verbal order patient squeezes the cuff at full power. Evaluation is done after three measurements. The average value is recorded. Explosive muscular strength was recorded as the maximum achieved numerical value expressed in mmHg. The values of static muscular strength was recorded as the time in which the respondent can maintain the achieved maximum value, while dynamic muscle strength measured time that the respondent hold his grip fist from the maximum value up to 80% of this value. Ring test measures oculomotor skilsl at the level of the shoulder joint. It consists of a rack with a stick length of 30–35 cm and 10 rings 5 times larger in diameter than the stick. A respondent has the task to put 10 rings on the stick as fast as possible. Each miss next to stick is recorded as a minus, and the total time is measured in seconds. O'Connor test also falls within the primary battery of special education tests, and it measures oculomotor skills at the level of the elbow joint. It consists of a plastic table measuring 16 × 22 cm with a large number of holes (diameters of 2.5 mm), and 10 plastic pegs (length 3.5 cm, thickness 2 mm with the head of 5 mm). The respondent has to put as fast as possible pegs in the holes. Pegs have to be placed with three fingers (thumb, index and middle finger). The time during which the respondent puts 10 pegs is measured by a stopwatch. Two attempts are allowed and better time is recorded. After evaluation each patient from the first experimental group gets his own personalized set of exercises chosen from an "open system of stimulation human development" 20. These exercises are the most common form of stimulation motor development in everyday special education clinical practice and they are conducted with the active participation of the patient and followed with vocalization. Each patient performs active psychomotor exercises on verbal instruction and if needed with demonstration by educator. In this study, exercises were from the group of exercises for stimulation the development of upper extremities general motor skills, exercises from the group for stimulating the development of coordination of movements at the level of the elbow and shoulder and from the group of exercises for stimulation the development of the power of certain movements [21]. Combined special education exercises as a supplement to occupational therapy treatment was applied to the first intervention group for 12 weeks, always at the end of each occupational therapy session, while the second intervention group was only in the process of occupational therapy. After that period participants from both groups were retested with the same tests.

The obtained data from the study were compared using two nonparametric tests – Mann-Whitney test and the Wilcoxon Signed Ranks Test. The Mann-Whitney test is used in experiments in which there are different subjects in each group, but the assumptions of parametric tests are not tenable. The Wilcoxon signed rank test has the null hypothesis that both samples are from the same population. The Wilcoxon test creates a pooled ranking of all observed differences between the two dependent measurements. It uses the standard normal distributed *z*-value to test of significance.

Data values are presented as the mean ± standard deviation and a p-value < 0.05 was considered as statistically significant. To compensate groups Student's t-test was used. Statistical analysis was performed with Statistical Package for the Social Sciences – IBM SPSS Statistics for Windows (version 15.0).

Results

The results obtained from hand grip tests are represented in Tables 1 and 2 and the results obtained from ring and O'Connor tests are quoted in the text below.

All the patients from the group who received special education exercises with occupational therapy were better in all measured variables – explosive muscular strength grip fist, static muscular strength grip fist and dynamic muscular

Table 1

Explosive, static and dynamic muscle strength grip fist (EMSGF, SMSGF and DMSGF, respectively) values for the right hand in both experimental groups before and after the treatment

Parameters	Before the treatment		After the treatment	
	SEOT group (n = 32)	OT group (n = 32)	SEOT group (n = 32)	OT group (n = 32)
EMSGF (mmHg)				
$\bar{x} \pm SD$	133.53 ± 34.93	152.25 ± 32.49	239.43 ± 41.45	183.45 ± 43.32
Mann-Whitney U test	557.500		148.000	
Wilcoxon W test	1187.500		725.000	
Z	-0.673		-5.186	
p	0.501		0.000	
SMSGF (mmHg)				
$\bar{x} \pm SD$	3.56 ± 1.94	3.32 ± 1.02	5.11 ± 2.00	3.59 ± 1.49
Mann-Whitney U test	533.500		245.500	
Wilcoxon W test	1163.500		800.500	
Z	-0.938		-3.412	
p	0.348		0.000	
DMSGF (mmHg)				
$\bar{x} \pm SD$	5.51 ± 3.43	5.06 ± 3.83	8.88 ± 3.35	6.55 ± 4.54
Mann-Whitney U test	580.500		312.000	
Wilcoxon W test	1210.500		840.000	
Z	-0.378		-3.136	
p	0.705		0.002	

SEOT group – intervention group receiving special education and occupational therapy;
OT group – intervention group receiving occupational therapy;
$\bar{x}$ – arithmetical mean; SD – standard deviation.

Table 2

Explosive, static and dynamic muscle strength grip fist (EMSGF, SMSGF and DMSGF, respectively) values for the left hand in both experimental groups before and after the treatment

Parameters	Before the treatment		After the treatment	
	SEOT group (n = 32)	OT group (n = 32)	SEOT group (n = 32)	OT group (n = 32)
EMSGF (mmHg)				
$\bar{x} \pm SD$	163.86 ± 47.00	141.34 ± 36.30	222.40 ± 41.53	160.42 ± 37.45
Mann-Whitney U test	562.500		277.500	
Wilcoxon W test	1192.500		907.500	
Z	-0.605		-3.818	
p	0.545		0.000	
SMSGF (mmHg)				
$\bar{x} \pm SD$	3.01 ± 1.85	2.78 ± 1.41	5.29 ± 2.00	3.02 ± 1.39
Mann-Whitney U test	508.500		241.000	
Wilcoxon W test	1138.500		913.000	
Z	-1.225		-4.262	
p	0.220		0.000	
DMSGF (mmHg)				
$\bar{x} \pm SD$	4.39 ± 4.94	4.13 ± 4.48	7.13 ± 3.84	4.97 ± 4.95
Mann-Whitney U test	557.500		312.000	
Wilcoxon W test	1187.500		840.000	
Z	-0.673		-3.136	
p	0.501		0.002	

SEOT group – intervention group receiving special education and occupational therapy;
OT group – intervention group receiving occupational therapy;
$\bar{x}$ – arithmetical mean; SD – standard deviation.

strength grip fist for both hands in hand grip test evaluation after the applied treatment. Achieved greater muscle strength of grip fist led to less errors and misses in catching and releasing rings while putting them on a stick during the execution performing the tasks from the test. The patients were also more accurate and finger movements were more refined in the act of holding rings (ring test) and in holding plastic pegs while putting them into holes (O'Connor test).

The results of the ring test which measures oculomotor skill at the level of shoulder for the right hand showed that the first intervention group which received special education exercises improved highly statistically significantly after the treatment compared to the second intervention group ($p = 0.000$), whereas before treatment both groups were equal ($p = 0.294$). The ring test for the left hand also showed differences between the groups on the basis of which it was

proved that the experimental group which received special education exercises highly statistically significantly improved after the treatment ($p = 0.000$) compared to the experimental group without exercises, while before the treatment both groups were equal ($p = 0.438$).

The results obtained in the O'Connor test which tested oculomotor skill at the level of elbow joint for right hand showed that the experimental group which received special education exercises improved highly statistically significantly after the treatment compared to the second intervention group which received only occupational therapy activities ($p = 0.000$), whereas before the treatment the two groups were equalized ($p = 0.668$). A statistical analysis in the O'Connor test for the left hand showed statistically significance differences between the intervention groups. The first experimental group improved highly statistically significantly after the treatment compared to the second experimental group which received only occupational therapy activities ($p = 0.000$), while before the treatment both groups were equal ($p = 0.321$).

The results showed a statistically significant improvement in the first experimental group of patients who were at different levels of progress in all tested variables of motor abilities, after the applied treatment, while participants in the second experimental group who received only occupational therapy activities had no such progress. The results of a repetitive upper extremity special education therapy program as a supplement to occupational therapy during inpatient rehabilitation resulted in a greater improvement in upper limb function in the group of patients who received special education exercises as a supplement to occupational therapy, than in the group who received only occupational therapy activities and this improvement showed a better voluntary arm control while performing movements in the shoulder and elbow that were required for the evaluation. Voluntary arm movements while performing shoulder flexion during the act of putting rings on a stick were made easier, smoother and faster. Although, it was not the subject of this study and therefore not recorded, attempts that all participants from the intervention group who received special education exercises made to re-establish the lost functions were not manifested only through physical efforts performed during the exercises, but they also invested a great amount of psychological effort, and each movement was strongly supported by their hopes and expectations. Even before the expiration of stipulated 12 weeks, while the special education treatment was still going on, more than half of the patients from this group reported better self confidence and felt more assertive while performing customary activities (particularly dressing and undressing).

Discussion

The obtained results are in accordance with other findings in the literature. Repeated motor activity (even very simple movements) is the basis of motor learning and recovery by causing changes in the cortex. For upper extremity motor training, the patients engaged in the task-specific activities usually achieve significantly greater gains compared to those who perform simple movements [22]. This would mean that constant repetition of practical tasks (direct and repeated training) as an alternative means of exercise can produce cortical changes that lead to improvement of functions. Ma and Trombly [23] argue that doing movements to achieve a certain objective during practical tasks can improve coordination and increase the range of motion. Most of the newer theories in the rehabilitation of stroke patients are based at least to some extent on the theory of motor learning [24]. Special education activities combined with individual exercise program and somatosensory stimulation in the form of repeated peripheral nerve stimulation, proved to be more effective in the chronic phase of recovery from stroke and in terms of improving the use of paretic hand in the Jebsen-Taylor test 25, 26. Another study confirmed that repeated exercises through the open and closed kinetic chain, with patients educated during rehabilitation, lead to the strengthening of extremity muscle strength and improve the balance in patients with stroke in the chronic phase of recovery, and was also a good predictor of functional recovery 27. On the contrary, French et al. 28 show that results of some studies prove that repetition of practical tasks in combination with intense exercise has no influence on the function of the upper extremities in patients with stroke, but slightly improved performance in activities of daily living. Some authors claim that hemiparetic upper extremities can increase strength and speed when a patient include self-vocalization while performing familiar motor tasks commonly used in occupational therapy 29. They also recommend organizing therapy sessions that simultaneously restore the speech and hand motor control that are so often impaired by stroke. Ostry et al. 30 identified the correspondence in the forms for speed hand movements and speech movements and suggested that the hand and speech can use the same elements of the motor programming. Neuroimaging observes that cortical motor area for programming speech, Broca's area, shows neurocortical activation during movements of the hand and wrist, and the same was observed when respondents are only imagined to perform the movement 29. Taking into account previous researches in this area, it is possible that repeated constant activation of Broca`s area in the execution of motor task can promote plasticity and lead to lasting improvements.

Limitations of the study and implications for practice

Although the obtained results show that the patients from the intervention group who received special education exercises achieved greater muscle grip strength and better coordination of upper extremities at the level of the elbow and the shoulder, the results must be interpreted with caution due to: relatively small sample, and almost perfect clinical settings in occupational therapy units provided at the Clinic for Rehabilitation "Dr. Miroslav Zotović" (the oldest rehabilitation center in Serbia with powerful tradition in rehabilitation people with post stroke hemiplegia). Therefore, the replication of the study with a larger sample and in different rehabilitation units is required. Also, the study lacks generalization in greater view. Because of the fact that each pa-

tient got personalized support through special education exercises that were added to standard occupational therapy treatment, thereby the duration of each therapy session extended, thus demanding more medical staff. Besides that, in the research there were no patients with a plegic (nonfunctional) hand who usually represent the major number of rehabilitation service users. It should be also acknowledged that only a limited number of arm movements were considered in this study, particularly when taking into account the complexity and numerous movements of the hand and fingers performed in the act of capturing and releasing different objects. And finally, after achieving greater muscle strength and increased mobility in the shoulder and the elbow joint, hand functions were not explored through object manipulation, neither patient's functional ability to use the paretic hand in everyday activities was further researched. Even though it was not an objective of this study, we consider it the greatest limitation of future implementation of special education exercises in clinical practice, because for each person it is more important what he/she can do with the hand than the numerical score obtained in test situation.

Conclusion

Patients with post-stroke conditions in a subacute phase of recovery have great potentials and they represent a group that is most likely to benefit from every intervention aimed at maximizing functional recovery. Therefore, further research in this area with a larger number of patients and in different institutions for rehabilitation is recommended and should be directed towards identifying instances of good practice.

For now, according to all the results obtained in this study it can be concluded that the aim of the research was fulfilled and the assumption confirmed that special education treatment as a supplement to occupational therapy activities have effects on improving upper extremities motor skills in adult patients with post-stroke hemiplegia included in the process of rehabilitation.

R E F E R E N C E S

1. *Johnston CS, Mendis S, Mathers CD.* Global variation in stroke burden and mortality: Estimates from monitoring, surveillance, and modelling. Lancet Neurol 2009; 8(4): 345−54.
2. *Furie KL, Kasner SE, Adams RJ, Albers GW, Bush RL, Fagan SC,* et al. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the american heart association/american stroke association. Stroke 2011; 42(1): 227−76.
3. *Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K,* et al. Heart disease and stroke statistics-2007 update: A report from the American Heart Association statistics committee and stroke statistics subcommittee. Circulation 2007; 115(5): e69−171.
4. *Kleindorfer D.* The bad news: Stroke incidence is stable. Lancet Neurol 2007; 6(6): 470−1.
5. *Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB,* et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 2012; 125(1): e2−e220.
6. *Canadian Institute for Health Information.* Canadian Institute for Health Information pilot project report: rehabilitation data standards for Canada. Ottawa: CIHI; 1999.
7. *Walker MF, Gladman JR, Lincoln NB, Siemonsma P, Whiteley T.* Occupational therapy for stroke patients not admitted to hospital: A randomized controlled trial. Lancet 1999; 354(9175): 278−80.
8. *De Wit L, Putman K, Schuback B, Komárek A, Angst F, Baert I,* et al. Motor and functional recovery after stroke: A comparison of 4 European rehabilitation centers. Stroke 2007; 38(7): 2101−7.
9. *Wolf SL, Thompson PA, Winstein CJ, Miller JP, Blanton SR, Nichols-Larsen DS,* et al. The EXCITE Stroke Trial. Comparing Early and Delayed Constraint-Induced Movement Therapy. Stroke 2010; 41(10): 2309−15.
10. *Krug G, McCormack G.* Occupational therapy: evidence-based interventions for stroke. Mo Med 2009; 106(2): 145−9.
11. *McPherson KM, Kersten P, McNaughton H, Turner-Stokes L.* Background to neurorehabilitation. In: *Candelise L, Hughes R, Liberati A, Uitdehag B, Warlow C,* editors. Management of neurological disorders: An evidence-based approach. Edinburgh: Blackwell Publishing; 2007. p. 100−9.
12. *Langhorne P, Coupar F, Pollock A.* Motor recovery after stroke: A systematic review. Lancet Neurol 2009; 8(8): 741−54.
13. *Harris JE, Eng JJ.* Paretic upper-limb strength best explains arm activity in people with stroke. Phys Ther 2007; 87(1): 88−97.
14. *Duncan PW, Sullivan KJ, Behrman AL, Azen SP, Wu SS, Nadeau SE,* et al. Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 2011; 364(21): 2026−36.
15. *Bobath B.* Adult Hemiplegia: evaluation and treatment. 3rd ed. Oxford: Butterworth Heinemann; 1990.
16. *Tyson SF, Selley AB.* The effect of perceived adherence to the Bobath concept on physiotherapists' choice of intervention used to treat postural control after stroke. Disabil Rehabil 2007; 29(5): 395−401.
17. *Barker RN, Brauer SG.* Upper limb recovery after stroke: The stroke survivors' perspective. Disabil Rehabil 2005; 27(20): 1213−23.
18. *Vučić R, Marković P, Savković N.* Clinical occupational therapy. 2nd ed. Belgrade: NIB Alternativa; 2001. (Serbian)
19. *Nudo RJ, Wise BM, Sifuentes F, Milliken GW.* Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science 1996; 272(5269): 1791−4.
20. *Stošljević M.* Basis of special education and rehabilitacion in people with motoric impairments. Belgrade: Društvo defektologa Srbije; 2013. (Serbian)
21. *Stošljević M, Nikič R, Eminović F, Pacić S.* Psychophysical handicap of children and youth. Belgrade: Društvo defektologa Srbije; 2013. (Serbian)
22. *Arya KN, Verma R, Garg RK, Sharma VP, Agarwal M, Aggarwal GG.* Meaningful task-specific training (MTST) for stroke rehabilitation: a randomized controlled trial. Top Stroke Rehabil 2012; 19(3): 193−211.
23. *Ma HI, Trombly CA.* A synthesis of the effects of occupational therapy for persons with stroke, Part II: Remediation of impairments. Am J Occup Ther 2002; 56(3): 260−74.
24. *Bowden MG, Woodbury ML, Duncan PW.* Promoting neuroplasticity and recovery after stroke: Future directions for rehabilitation clinical trials. Curr Opin Neurol 2013; 26(1): 37−42.
25. *Dos Santos-Fontes RL, Ferreiro de Andrade KN, Sterr A, Conforto AB.* Home-based nerve stimulation to enhance effects of motor training in patients in the chronic phase after stroke: a

proof-of-principle study. Neurorehabil Neural Repair 2013; 27(6): 483−90.
26. *Klaiput A, Kitisomprayoonkul W*. Increased pinch strength in acute and subacute stroke patients after simultaneous median and ulnar sensory stimulation. Neurorehabil Neural Repair 2009; 23(4): 351−6.
27. *Lee NK, Son SM, Nam SH, Kwon JW, Kang KW, Kim K*. Effects of progressive resistance training integrated with foot and ankle compression on spatiotemporal gait parameters of individuals with stroke. J Phys Ther Sci 2013; 25(10): 1235−7.
28. *French B, Thomas LH, Leathley MJ, Sutton CJ, Mcadam JA, Forster A*, et al. Repetitive task training for improving functional ability after stroke. Stroke 2009; 40(4): 98−9.
29. *Maitra KK, Telage KM, Rice MS*. Self-speech-induced facilitation of simple reaching movements in persons with stroke. Am J Occup Ther 2006; 60(2): 146−54.
30. *Ostry DJ, Cooke JD, Munhall KG*. Velocity curves of human arm and speech movements. Exp Brain Res 1987; 68: 37−46.

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Quality of life of treated opiate addicts in the methadone maintenance program and those treated with buprenorphine

Kvalitet života lečenih opijatskih zavisnika u programu metadonskog održavanja i zavisnika lečenih buprenorfinom

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Abstract

Background/Aim. Although the characteristics of the treatment are the most researched determinants of quality of life of opiate addicts, it is indisputable that there is a certain influence of the characteristics of addicts and addiction, too. The aim of this study was to determine which addicts characteristics, as well as the characteristics of the addiction and treatment have predicative influence on the quality of life of the opiate addicts treated in the methadone maintenance program and those treated with buprenorphine. **Methods.** The epidemiological cross-sectional study was carried out in 2013 at the Clinical Center Niš, on a total of 64 opiate addicts, both sexes, aged 18 and older (32 addicts in the methadone program, chosen by random selection, and 32 addicts treated with buprenorphine, matched by sex and age). Necessary data were collected in a "face to face" interview with the examinees, based on the autonomous kind of a questionnaire, together with the use of the standardized World Health Organization (WHO) instruments: for health status, for the level of severity of addiction and for the quality of life measuring) based on which the health index (EQ-5D), Addiction Severity Index (ASI) and the quality of life index (WHOQOL-BREF) were calculated. The data were described by the methods of descriptive statistics, while the differences between groups were analyzed by applying χ^2 and *t*-test. Multiple regressions were used to determine the predictors. **Results**. The addicts in the methadone program showed much worse perception of quality of life than those treated in another way, although, according to the values of quality of life, they did not differ significantly. The most numerous predictors of the level of quality of life were health characteristics, characteristics of the socioeconomic position of the examinees, as well as different consequences of addiction. The influence of treatment was less noticeable. Participating in the methadone program had predicative influence on perception and the level of quality of life of the addicts in mental area and that of the environment. The influence of the characteristics of methadone treatment in physical and social area was insignificant. **Conclusion**. Variations in the perception and level of the quality of life of opiate addicts in different areas cannot be explained using only one predictor. The number of determining variables is large, and its impact complex.

Key words:
opioid-related disorders; addiction; opiate substitution treatment; methadone; buprenorphine; quality of life; treatment outcome.

Apstrakt

Uvod/Cilj. Iako su karakteristike lečenja najčešće istraživane determinante kvaliteta života opijatskih zavisnika, nesporno je da izvestan uticaj imaju i karakteristike zavisnika i zavisnosti. Cilj rada bio je da se utvrdi koje karakteristike zavisnika, zavisnosti i lečenja imaju prediktivni uticaj na kvalitet života opijatskih zavisnika u programu metadonskog održavanja i zavisnika lečenih buprenorfinom. **Metode**. Ova epidemiološka studija preseka sprovedena je na uzorku od 64 zavisnika od opijata oba pola, uzrasta 18 i više godina (32 zavisnika u programu metadonskog održavanja, izabranih metodom slučajnog odabira i 32 zavisnika lečenih buprenorfinom, odabranih prema polu i starosti), koji su lečeni u Kliničkom centru u Nišu. Potrebni podaci sakupljeni su intervjuom sa ispitanicima na bazi samostalno razvijenog upitnika, uz korišćenje standardizovanih instrumenata Svetske zdravstvene organizacije (WHO) za merenje zdravstvenog statusa, ozbiljnosti posle-dica zavisnosti, i kvaliteta života, na osnovu čega su izračuna-vani: indeks zdravlja (EQ-5D), indeks težine posledica zavisno-sti (ASI) i indeks kvaliteta života (WHOQOL-BREF). Podaci su opisani metodama deskriptivne statistike; razlike između grupa analizirane su primenom χ^2 i *t*-testa. Za izdvajanje prediktora korišćena je multipla regresija. **Rezultati.** Zavisnici u metadonskom programu imali su značajno lošiju percepciju kvaliteta života od zavisnika lečenih buprenorfinom, iako se nisu razlikovali značajno od njih prema indeksu kvaliteta života. Najbrojniji prediktori kvaliteta života bili su karakteristike socijalnoekonomske pozicije ispitanika, zdravstvene karakteristike, kao i težina posledica zavisnosti. Uticaj lečenja bio je

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manje izražen. Učešće u metadonskom programu ima prediktivni uticaj na percepciju i indeks kvaliteta života zavisnika u psihičkom domenu i domenu okruženja. Najveći prediktivni značaj imali su trajanje i prekidi lečenja metadonom. Uticaj karakteristika metadonskog lečenja u fizičkom i socijalnom domenu bio je marginalan. **Zaključak** Varijacije u percepciji i nivou kvaliteta života opijatskih zavisnika u različitim domenima ne mogu se objasniti jednim prediktorom. Broj determinišućih varijabli je veliki, a dejstvo kompleksno.

Ključne reči:
poremećaji izazvani opioidima; opijati, supstituciona terapija; metadon; buprenorfin; kvalitet života; lečenje, ishod.

Introduction

The substitution treatment of opioid addictions can increase the quality of life of addicts [1] and reduce the Addiction Severity Index (ASI), but it is not yet clear what determines these changes [2]. The quality of life is the perception of respondents about the condition, functioning and satisfaction with various aspects in all or selected areas of life; reflects the standards, norms and expectations of the respondents in terms of the quality of life. Variations are usually associated with different treatment characteristics (length, doses, the content of treatment, psychosocial support), as well as the characteristics of health care in general and addiction characteristics.

Most researchers agree that treatment can improve the quality of life of addicts, regardless of whether it is based on the substitution of methadone or buprenorphine [3], but they point out that the effect of treatment is not equal in all the domains of quality of life. Some point out that the methadone treatment produces the greatest effect in the psychological domain [4], the others in physical and social domain [5], and the third in the physical, psychological and the domain of environment [6–8]. Some researchers point out that methadone treatment produces significant improvements in quality of life in all four domains [9] resulting from the cumulative effect of the treatment, since the improvement in one, affects the changes in other domains of quality of life [10].

The duration of treatment is the most researched factor. Most researchers agree that the effects of methadone substitution are the highest in the first three months of treatment [4, 5], although this program has certain effects thereafter. Moreover, a large number of researchers point to the negative effects of methadone treatment in the various domains of quality of life [11–15].

The quality of life is emphasized as an important predictor of progress.

Among all determinants, only the quality of life before treatment and its early changes are statistically significantly related to remaining in the treatment at least 6 months [16]. The addicts' quality of life, before entering the treatment program is an important predictor of an early progress in longer remaining in the program of methadone sustaining [16–18]. Others point out that continuous treatment oriented to the needs of individual patients [19] is statistically significantly associated with the cease of being an addict and is the strongest predictor of changes in the quality of life [18], with significant effects on the psychological and social functioning [10].

Nearly all of the researchers agree that the changes in the quality of life may be associated with dosing [2] and that the higher dose of methadone has a greater potential to increase the quality of life and reduce undesired events during the treatment [18, 20].

The researchers point out that a certain contribution to the variations have other characteristics of addicts (demographic, socioeconomic, healthy), as well as the characteristics of addiction, but stress that their influence is sometimes difficult to separate from the contributions of the treatment.

The impact of demographic characteristics on the quality of life of drug users is difficult to separate from the contribution of other variables [21–23]. The effect of age on the quality of life of addicts in the physical domain is predictive, but it is stressed that it must be viewed in conjunction with variables such as employment, comorbidity, hospitalization, abuse, the age of first drug use [24]. Socioeconomic position is an important predictor of the quality of life of drug users [10, 21, 22, 24]. It is described as the entire set of variables, such as education, employment, family life [1, 10, 21, 22.]

The influence of the environment on the quality of life level differences in the addicts included in the methadone program is often less direct, more often within the scope of many other personal factors, factors that are related to drugs and/or treatment, so it is difficult to specify it. Variations in the quality of life are described in relation to the environment influence, financial status, free time, and social activities, rather than direct heroin influence [12].

The most important predictor of the quality of life is inability of the addicts to adapt to some new life situation, bad life conditions and the absence of permanent residence as a predictor of low quality of life of the addicts [18].

The researchers point out the importance of the family, household and the relationships within, in women who have kids there is a downfall in the areas of social support, psychological and environmental domain, as well as performance [21, 22], and the importance of life conditions and social support, which especially becomes prominent when there are symptoms and trauma (violence towards women) [25].

The influence of physical and sexual harassment on the differences in the quality of life in psychological domain is something that researchers confirmed [24].

There is an obvious protective impact of the scope of social network and the existence of close friends ready to give support to an addict. Providing social support to the addicts causes great increase in the quality of life [12, 13].

Health features of the addicts are the important determining factor of the quality of life. In that sense, it is important to point out the influence of comorbidity, chronic disease, psychological distress, depression, HIV infection on the quality of life in some domains [26]. Some variations in the le-

vel of quality of life, especially physical and psychological domain, can be explained by the presence of the symptoms and traumas [25]. Use of alcohol (the amount taken in the last 30 days) can explain some variations in the environmental domain. The intensity of psychological distress and taking psychological treatment medications are associated with low quality of life [12]. Human immunodefitiency virus (HIV)+ addicts have substantially lower quality of life scores in the areas of physical health and functional abilities, as well as quality of life, related to health in general [26].

There is a high level of agreement around the addiction characteristics (the type of the drugs used, the number of taken substances, their combining, the length of drug abuse period) which an addict brings into the treatment, as a significant factor of the present quality of life and the changes which are expected. Some researchers confirmed the differences in the outcome of male and female addicts, which could be related to specific characteristics of opiate use and the initial treatment [23]. The age at the moment of the first drug abuse (an injection episode) is significantly related to the quality of life in physical domain [24]. Sedative abuse, cocaine use, the duration of cocaine use, as well as the use of the great number of substances in the last month, are much related to the quality of life in psychological domain. However, everyone claims that variations in the physical and psychological domain of the quality of life can not be explained merely by drug effects. There is no direct effect of heroin use on the quality of life [13–20]. Drug effect is felt in coeffects with some other (personal and environmental) characteristics (life circumstances, financial status, changes, perspective, taking part in some free and social activities, family and friends support) [19].

The treatment effect has often been the subject of researching the quality of life of the drug addicts. The research in opiate addicts is focused on clinical efficacy of the treatment more than the quality of life assessed from the perspective of drug users.

The aim of this study was to identify the differences in quality of life between different groups of drug users (opiate addicts in methadone maintenance program and those treated with buprenorphine) that can be correlated with the treatment and to investigate what demographic, socioeconomic and health characteristics of the respondents and their environment, *ie* what characteristics of addiction and treatment determine the differences.

Methods

This epidemiological cross-sectional study was performed in July and December 2013 in accordance with the norms of the Helsinki Declaration and with the approval of the Ethics Committee of the Faculty of Medical Sciences in Kragujevac. The observed groups were addicted to opiates, of both genders, aged 18 and older, with different (demographic, socioeconomic and healthy) characteristics, which were treated at the Clinic for Mental Health at the Clinical Center Niš. Using G Power program and power parameters of the study for medium power impact, the required number of units of observation was determined. On completion of the stratification of addicts according to the method of treatment (methadone *vs* buprenorphine) from each stratum was selected a simple random sample using the table of random numbers. The stratified sample had 64 participants, divided into two groups.

The group A included 32 subjects involved in the methadone program [17 (53.12%) males, and 15 (49.88%) females, mean age 48.12 ± 3.15], and the group B of 32 subjects treated with buprenorphine [13 (40.62%) males, 19 (59.38%) females, mean age 46.94 ± 4.56]. The groups were homogeneous in age ($p = 0.233$) and gender ($p = 0.452$).

The instrument to collect data on the characteristics of the respondents was the sociodemographic questionnaire. Through interviews "face-to-face" with respondents in a medical institution, we collected data on the following issues: demographic, socioeconomic and health characteristics (gender, age, level of education, employment, marital status, children, the number and type of the disease), characteristics of the environment (housing conditions, living conditions, family characteristics and family functioning, the number of friends and relationships with friends), characteristics of health care (the choice of a doctor, the use of primary and preventive care, participation in methadone treatment, duration and type of methadone treatment, continuity and treatment interruption).

The instrument to collect data on health status (quality of life related to health) was a standardized questionnaire to measure the health status of the World Health Organisation (WHO) (WHO EQ-5D, Version 4.0; 2011). This study used both questionnaire modalities – information system (EQ-5D-5L), which focuses on five dimensions of health (mobility, self-protection, the usual activities, the presence of pain/discomfort and anxiety/depression) and visual analogue scale (EQ-5D-VAS) to which the respondent marked the assessment of the health of the worst possible to the best possible level [27]. Euro QOL EQ-5D index, that represents quantitative measures of treatment outcome of health care and aggregate measures of health and quality of life was calculated on the basic of the created data [28].

To register the seriousness of the addiction consequences in seven areas (health, professional, social, family, legal, addictive and psychiatric) functioning in the last 30 days and during the entire life of the respondents, questionnaire Addiction Severity Index (ASI) was used [29].

The overall quality of life in this study was expressed by the perception of quality of life that was measured in 4 different domains (physical functioning, psychological functioning, social functioning, environment).

The instrument for collecting data on the quality of life was a standardized questionnaire to calculate the WHOQOL-BREF index [30] which measures the overall quality of life on the basis of respondents assessment, that contributed with their standards, norms and expectations.

The quality of life was shown as the frequency of distribution of respondents by the category of perception of quality of life graphically, parallel to both groups. To determine the significance of differences, χ^2 test was used.

The level of quality of life in different domains was described by descriptive statistical parameters (mean, standard deviation, median, minimum and maximum value). Data on the level of quality of life in different domains were presented in tables, parallel to both groups. To determine the significance of differences *t*-test was used.

The focus of statistical analysis were the determinants of differences in the perception and the quality of life of opiate addicts among the characteristics of the respondents, their health and health care, environmental characteristics, depending on the characteristics and treatment.

For testing and isolation of potential factors of importance for the perception of the level of quality of addicts life, multiple linear regression was used. In the analysis we used SPSS 17.0 for Windows.

Results

The addicts in the methadone program had much poorer perception of quality of life than the addicts treated with buprenorphine. The differences between the two groups were statistically significant [(χ^2 = 29.86 degrees of freadom (DF) = 8 p = 0.000)].

Analysis of the predictors of the perception of quality of life (R^2 = 0.929 standard eror (SE) = 0.584 F = 24.250 p = 0.016) determined the predictive value of demographic (gender), socioeconomic (level of education, type of settlement) and health characteristics of respondents describing functional status (mobility, self-care, the ability to perform everyday activities), the presence of symptoms (irritability/depression) and the perception of health and the characteristics of the household in which they lived (number of household members, number of children). Among the characteristics of treatment, only the duration of methadone treatment had the predictive value (Table 1).

The respondents who lived in a household with more members and fewer children, were more educated and the males who lived in the city better perceived their quality of life. A directly proportional correlation between health status (functional capacity indicated by the frequency of the problem in terms of mobility and the ability to care for themselves, the presence of limitations in their daily activities, often signs of irritability and depression) in this study perceived better their quality of life. The overall quality of life is perceived poorer by respondents with higher ASI (the presence of severe consequences of addiction). The overall quality of life was perceived better by respondents involved in the methadone maintenance program. The higher index of quality of life in the domain of physical health can be expected in patients with fewer expressed addicts consequences (lower ASI) and a shorter time length of being an addict, just as the other researchers claim. In the methadone program addict group we established a bit higher values of the level of quality of life in the domain of physical health and environment, than in the out-of-program addicts. With the addicts off the methadone program, higher values of quality of life in the domain of physical health and social interactions were more noticeable. The described differences in the level of quality of life between the examined groups were not statistically significant in any domain (Table 2).

Analysis of the predictors of quality of life in the field of mental health revealed a predictive value of demographic (gender), socioeconomic (level of education, type of occupation, monthly income, type of settlement in which they live), health characteristics, which describe the functional status of the respondents (mobility, ability of self-care), the presence of symptoms (pain/discomfort, irritability/depression) *ie*, perception of health, health care, especially continuity (the number of visits to general practice – GP) and household characteristics (household size, building in which they live, the size of the living unit, the way of heating) (R^2 = 1.000 SE = 0.151 F = 572.779 p = 0.033).

Analysis of the predictors of quality of life in the field of mental health aligned characteristics of addiction, as well as ASI indices and the length of heroin abuse – the length of taking drugs.

Characteristics of addiction treatment that showed predictive impact on the quality of life in the field of mental health are the length of methadone treatment and interruptions in methadone treatment (Table 3).

A higher index of quality of life in the domain of physical health could be expected in patients who live in smaller apartments heated by solid fuel stoves, in small towns (villages), have no sedentary job, have incomes above the minimum, live in or out of wedlock, have higher educational attainment, have better functional ability (and fewer problems in performing daily activities) and perceive their health better, pay visits to the doctor and have regular health controls. A higher quality of life in the physical domain was revealed in addicts with lower ASI (with fewer expressed addicts consequences) and shorter period of being addicts (Table 3).

Analysis of the predictors of quality of life in the field of mental health (R^2 = 1.000 SE = 0.236 F = 360,767 p = 0.042) showed a predictive value of demographic (gender), socioeconomic (level of education, type of occupation, monthly income, type of settlement in which they live), health characteristics, which describe the functional status of the respondents (mobility, ability of self-care), the presence of symptoms (pain/discomfort, irritability/depression) *ie*, perception of health, health care, especially continuity (the number of visits to GP) and household characteristics (household size, the building in which they live, the size of the living unit, the way of heating. Analysis of the predictors of quality of life in the field of mental health aligned characteristics of addiction, as well ASIs describing different effects and consequences of addiction, except the effects on employment, and the length of heroin abuse – the length of taking drugs).

Characteristics of addiction treatment that showed a predictive impact on the quality of life in the field of mental health are the length of methadone treatment and interruptions in methadone treatment (Table 3).

The higher index of quality of life in the psychological domain was found in the male addicts with higher education, higher income and more profitable occupation, those who

Table 1

Predictors of perception of the quality of life of opiate addicts

Predictors	Addicts n (%)	Quality of life perception				
		very bad n (%)	bad n (%)	acceptable n (%)	good n (%)	very good n (%)
Gender						
male	30 (46.9)	2 (6.67)	5 (16.67)	10 (33.33)	11 (36.66)	2 (6.67)
female	34 (53.1)	4 (11.76)	13 (38.24)	14 (41.18)	3 (8.82)	0 (0.00)
β (*p*)				-1.753 (0.007)		
Education						
primary education	15 (23.4)	3 (20.00)	6 (40.00)	5 (33.33)	1 (6.67)	0 (0.00)
moderate (3 and 4 year)	46 (71.9)	3 (6.52)	12 (26.09)	18 (39.13)	12 (26.09)	1 (2.17)
more and higher	3 (4.7)	0 (0.00)	0 (0.00)	1 (33.33)	1 (33.33)	1 (33.33)
β (*p*)				1.403 (0.024)		
Number of children						
without	26 (40.6)	2 (7.69)	4 (15.39)	10 (38.46)	8 (30.77)	2 (7.69)
one	25 (39.1)	3 (12.00)	8 (32.00)	9 (36.00)	5 (20.00)	0 (0.00)
two or more	13 (20.3)	1 (7.69)	6 (46.16)	5 (38.46)	1 (7.69)	0 (0.00)
β (*p*)				-0.808 (0.047)		
Number of household members						
two	12 (18.7)	2 (16.67)	5 (41.67)	4 (33.33)	1 (8.33)	0 (0.00)
three	21 (32.8)	2 (9.52)	9 (42.86)	7 (33.33)	3 (14.29)	0 (0.00)
four	20 (31.2)	1 (5.00)	3 (15.00)	10 (50.00)	5 (25.00)	1 (5.00)
five or more	11(17.3)	1 (9.09)	1 (9.09)	3 (27.27)	5 (45.45)	1 (9.09)
β (*p*)				4.245 (0.011)		
Type of settlement						
village	8 (12.5)	1 (12.50)	4 (50.00)	3 (37.50)	0 (0.00)	0 (0.00)
settlement	30 (46.9)	3 (10.00)	11 (36.67)	11 (36.67)	5 (16.66)	0 (0.00)
town	26 (40.6)	2 (7.69)	3 (11.54)	10 (38.46)	9 (34.62)	2 (7.69)
β (*p*)				0.981 (0.035)		
Medical Composite Score						
good (5)	44(68.8)	5 (11.36)	13 (29.54)	17 (38.64)	9 (20.46)	0 (0.00)
mediocre (6–10)	15(23.4)	1 (6.67)	4 (26.66)	6 (40.00)	3 (20.00)	1 (6.67)
bad (> 10)	5 (7.8)	0	1	1	2	1
β (*p*)				-2.694 (0.011)		
Index of quality of life (EQ-5D-VAS)						
bad (50)	15 (23.4)	3 (20.00)	6 (40.00)	4 (26.67)	2 (13.33)	0 (0.00)
mediocre (50–75)	17 (26.6)	2 (11.76)	7 (41.18)	6 (35.3)	2 (11.76)	0 (0.00)
good (75 and more)	32 (50.0)	1 (3.12)	5 (15.62)	14 (43.75)	10 (31.26)	2 (6.25)
β (*p*)				-1.602 (0.014)		
ASI / Depending on the severity of consequences						
the easiest 20)	20 (31.3)	2 (10.00)	4 (20.00)	7 (35.00)	6 (30.00)	1 (5.00)
moderately (20–30)	26 (40.6)	2 (7.69)	8 (30.76)	9 (34.62)	6 (23.08)	1 (3.85)
the hardest (30 and more)	18 (28.1)	2 (11.11)	6 (33.33)	8 (44.45)	2 (11.11)	0 (0.00)
β (*p*)				-2.672(0.015)		
Duration of methadone treatment						
out of treatement (group B)	32 (50.0)	3 (9.37)	6 (18.75)	14 (43.75)	7 (21.88)	2 (6.25)
up to 24 months	5 (7.8)	0 (0.00)	4 (80.00)	1 (20.00)	0 (0.00)	0 (0.00)
24–48 months	16(25.0)	2 (12.50)	6 (37.50)	4 (25.00)	4 (25.00)	0 (0.00)
48–72 months	8(12.5)	1 (12.50)	2 (25.00)	3 (37.50)	2 (25.00)	0 (0.00)
through 72 months	3 (4.7)	0 (0.00)	0 (0.00)	2 (66.67)	1 (33.33)	0 (0.00)
β (*p*)				2.977 (0.007)		

β – regression coefficient; *p* – probability; ASI – Addiction Severity Index.

Table 2

Descriptive statistical parameters of the quality of life index

Category	Descriptive statistical parameters						
	x̄	SD	CV (%)	Med	SE	Min	Max
Physical health							
group A	23.72	3.57	15.05	24	0.63	16	32
group B	22.12	3.35	15.14	22	0.593	15	29
Mental health							
group A	18.78	4.35	23.16	19	0.768	10	27
group B	20.53	4.41	21.48	20.5	0.780	10	30
Social relations							
group A	9.40	2.82	30.00	9.5	0.497	4	15
group B	10.22	2.35	22.99	10	0.416	6	15
Environment							
group A	28.03	4.77	17.02	27.50	0.843	17	38
group B	27.44	4.75	17.31	27.00	0.839	21	39

Group A – addicts in methadone treatment; Group B – addicts treated with buprenorphine; x̄ – mean value; SD – standard deviation; CV – coefficient of variation; Med – median; SE – standard error; Min – minimal; Max – maximal.

Table 3

Predictors of the quality of life of opiate addicts

Predictors	Addicts n (%)	Quality of life domains, n (%)			
		mental health	physical health	social relations	environment
Gender					
male	30 (46.9)	24.32 (2.17)	21.03 (3.41)	10.07 (1.35)	27.781 (3.75)
female	34 (53.1)	21.72 (3.57)	19.81 (3.31)	9.99 (2.82)	28.11 (3.77)
β (*p*)		-1.031 (0.015)	-1.334 (0.014)	-	-
Education					
primary	15 (23.4)	21.12 (3.35)	19.53 (4.41)	9.92 (2.92)	27.91 (4.75)
intermediate (3 and 4 years)	46 (71.9)	22.43 (3.22)	21.08 (4.35)	10.36 (2.44)	28.03 (4.77)
more and higher	3 (4.7)	23.71 (3.57)	22.21 (4.26)	10.41 (2.25)	28.73 (4.41)
β (*p*)		1.053 (0.018)	1.269 (0.019)	-	-
Interest					
managers officials	7 (10.9)	22.84 (3.35)	20.97 (4.41)	10.02 (2.35)	27.66 (3.75)
VKV, KV workers	36 (56.2)	23.78 (3.57)	20.41 (4.35)	10.40 (2.82)	28.03 (3.77)
PKV, NKV workers	17 (26.6)	19.92 (3.35)	19.33 (4.41)	10.32 (2.35)	27.94 (3.61)
Housewives, students, unable to work	4 (6.3)	17.72 (3.57)	18.78 (4.35)	9.91 (1.82)	28.12 (3.97)
β (*p*)		-1.061 (0.018)	-0.815 (0.029)	-	-
Monthly income per member of the family household					
without receiving	19 (30.16)	21.37 (3.25)	18.22 (4.22)	9.31 (2.36)	27.74 (3.55)
minimum	27 (42.86)	23.72 (3.52)	18.78 (4.25)	9.40 (2.81)	28.01 (3.57)
above the minimum income	17 (26.98)	23.92 (3.15)	20.13 (4.11)	10.01 (2.30)	28.13 (3.71)
β (*p*)		0.990 (0.029)	0.723 (0.049)	-	-
Marital status					
unmarried	24 (37.5)	21.01 (2.93)	19.76 (4.17)	9.19 (2.37)	28.94 (4.75)
divorced	14 (21.9)	22.22 (3.57)	18.98 (4.35)	9.90 (2.82)	27.03 (4.77)
married/extramarital community	26 (40.6)	23.92 (3.35)	20.01 (4.41)	10.12 (2.35)	26.85 (3.42)
β (*p*)		0.784 (0.028)	-		-1.306 (0.027)
Number of household members					
to two members	12 (18.75)	22.52 (3.38)	17.78 (4.35)	9.01 (2.28)	27.97 (4.22)
3 members	21 (32.81)	23.22 (3.70)	20.03 (4.54)	10.32 (2.23)	28.49 (4.52)
4 members	20 (31.25)	22.91 (3.51)	20.21 (4.21)	10.98 (2.35)	29.02 (4.38)
5 and more	11 (17.19)	23.64 (3.05)	20.53 (4.23)	10.99 (2.68)	28.22 (4.41)
β (*p*)		-	4.896 (0.018)	2.139 (0.033)	-
Type of settlement					
village	8 (12.5)	23.92 (3.57)	18.07 (4.14)	9.81 (2.39)	27.03 (4.75)
settlement	30 (46.9)	22.87 (3.32)	18.78 (4.05)	9.40 (2.82)	28.03 (4.27)
town	26 (40.6)	22.02 (3.71)	20.23 (4.41)	10.22 (2.35)	29.10 (4.14)
β (*p*)		-0.444 (0.046)	0.942 (0.027)	-	0.905 (0.035)
Condition of house					
house with garden	32 (50.0)	22.61 (3.39)	18.31 (4.32)	10.21(2.47)	28.23 (4.76)
Apartment	32 (50.0)	23.72 (3.27)	20.28 (4.31)	9.90 (2.81)	27.74 (4.65)
β (*p*)		-	1.109 (0.030)	-	-
Surface area in which they live					
20 m^2	41 (64.0)	24.08 (3.34)	18.52 (4.18)	10.22 (2.35)	27.44 (4.75)
21–40 m^2	14 (21.9)	23.72 (3.57)	18.78 (4.35)	9.40 (2.82)	28.03 (4.77)
41 and more m^2	9 (14.1)	22.12 (3.35)	20.50 (4.41)	10.07 (2.11)	27.99 (4.82)
β (*p*)		-0.543 (0.048)	0.976 (0.034)	-	-
Warming up the space in which they live					
steam. central or floor heating	20 (31.2)	22.12 (3.35)	18.62 (4.45)	10.22 (2.35)	27.44 (4.75)
furnaces electric	19 (29.7)	23.71(3.57)	18.78 (4.35)	9.40 (2.82)	28.03 (4.77)
stoves	25 (39.1)	24.43 (3.32)	20.53 (4.41)	9.92 (2.19)	27.93 (4.56)
β (*p*)		0.625 (0.042)	0.850 (0.039)	-	-
Health status / Functional status (Rank)					
good (up to 5)	45 (70.3)	24.59 (3.39)	20.53 (4.41)	10.92 (2.35)	27.44 (4.75)

acceptable (6–10)	14 (21.9)	23.72 (3.57)	18.78 (4.35)	9.40 (2.82)	28.03 (4.77)
bad (more than10)	5 (7.8)	22.12 (3.35)	18.37 (4.15)	9.05 (2.73)	27.99 (4.76)
β (*p*)		-0.588 (0.013)	-3.028 (0.017)	-1.815 (0.024)	-
Health index/perception of health (EQ-5D-VAS)					
bad (50)	15 (23.4)	22.03 (3.35)	20.53 (4.41)	10.22 (2.35)	27.44 (4.51)
acceptable (50–75)	17 (26.6)	23.24 (3.21)	18.78 (4.35)	9.40 (2.82)	28.03 (4.36)
good (75 and more)	32 (50.0)	24.19 (3.70)	18.50 (4.43)	9.45 (3.02)	28.21 (4.45)
β (*p*)		1.078 (0.028)	-0.795 (0.049)	-	-
Number of general practitioner visits					
up to three times	34 (53.1)	21.90 (3.45)	19.58 (4.15)	10.22 (2.35)	27.11 (4.71)
4–6	16 (25.0)	23.62 (3.70)	20.32 (4.01)	9.40 (2.82)	28.03 (4.27)
7 and more	14 (21.9)	23.98 (3.35)	21.53 (4.21)		29.29 (4.44)
β (*p*)		0.948 (0.026)	0.649 (0.049)	-	1.443 (0.028)
The reason for visits to general practitioners					
health check	13 (20.3)	24.19 (3.39)	18.97 (4.41)	10.12 (2.34)	27.44 (4.15)
prescribing drugs	24 (37.5)	23.72 (3.51)	20.03 (4.35)	9.49 (1.82)	28.03 (4.07)
administration	27 (42.2)	22.06 (3.33)	19.89 (4.35)	10.01 (4.22)	27.90 (4.43)
β (*p*)		-1.091 (0.026)	-	-	-
How long they have been addicts					
up to 5 years	9 (14.1)	23.92 (3.57)	18.78 (4.01)	10.12 (2.31)	26.91 (4.46)
5–10	21 (32.8)	23.12 (3.35)	19.53 (4.35)	9.80 (2.28)	28.11 (4.32)
10–15	19 (29.7)	22.06 (3.57)	20.26 (3.41)	10.04 (2.56)	28.94 (4.25)
16 and more	15 (23.4)	21.22 (3.35)	20.88 (2.35)	9.96 (2.78)	28.99 (4.47)
β (*p*)		-0.724 (0.023)	0.503 (0.041)	-	0.651 (0.038)
ASI / Depending on the severity of consequences					
the easiest 20)	20 (31.3)	24.38 (3.57)	20.65 (4.41)	10.22 (2.35)	28.13 (4.77)
acceptable(20–30)	26 (40.6)	22.02 (3.35)	18.77 (4.35)	9.39 (2.82)	27.44 (4.75)
the hardest (30 and more)	18 (28.1)	21.55 (3.08)	18.01 (4.33)	8.12 (2.23)	26.01 (3.92)
β (*p*)		-1.255 (0.013)	-1.079 (0.018)	-0.870 (0.020)	-1.850 (0.039)
Duration of methadone treatment					
out of treatment (group B)	32 (50.0)	22.12 (3.35)	20.53 (4.41)	10.22 (2.35)	27.44 (4.75)
Up to 24	5 (7.8)	23.97 (3.17)	18.32 (4.10)	8.89 (2.22)	28.33 (4.34)
24–48	16 (25.0)	23.89 (3.25)	18.69 (4.19)	9.47 (2.51)	27.38 (4.72)
48–72 months	8 (12.5)	23.59 (3.07)	19.02 (4.34)	9.35 (2.66)	28.83 (4.56)
trough 72 months	3 (4.7)	22.73 (3.03)	19.37 (4.52)	10.02(2.72)	28.67 (3.92)
β (*p*)		-	2.310 (0.022)	-	-
Interruptions in treatment					
yes	16 (21.9)	23.11 (2.31)	20.93 (4.41)	10.02 (2.35)	28.53 (4.07)
no	48 (78.1)	23.92 (2.57)	18.81 (4.35)	9.76 (2.82)	26.54 (4.25)
β (*p*)		-	-0.755 (0.025)	-	-0.892 (0.027)

β – regression coefficient; *p* – probability; ASI – Addiction Severity Index.

live in the city, in larger houses heated by solid fuel stoves, surrounded by a large number of household members. The quality of life in the psychological domain is higher in drug addicts with fewer problems with taking care of themselves, with less frequent symptoms such as pain and discomfort, even when they perceive their health as poor, if they behave protectively (and often pay a visit to a doctor). A higher quality of life in the field of mental health was found in those with longer period of being addicts, with fewer consequences (lower ASI index), who were involved in the methadone program which they rarely abandoned.

Analysis of the predictors of quality of life in the domain of social relations (R^2 = 0.992 SE = 1.370 F = 4.402 p = 0.036) determined the predictive value of functional status (ability to self-care) and environment characteristics (number of household members). Except as noted, the predictive value of other characteristics (demographic, socioeconomic, healthy characteristics of respondents and characteristics of their health care) were not determined.

The predictors of quality of life in the domain of social health aligned the consequences of addiction, too (ASI indexes).

Characteristics of treatment have no predictive effect on quality of life in the domain of social relations (Table 3).

A higher level of quality of life in the domain of social health can be expected in patients with a lower ASI, with no functional limitations, who live in a household with more members.

Analysis of the predictors of quality of life in the domain of environment (R^2 = 0.983 SE = 3.498 F = 1.884 p = 0.050) determined the predictive importance of the characteristics of the socioeconomic position of the respondents (ma-

rital status, type of settlement in which they live), characteristics that describe the health care of patients, especially continuity (the number of visits to the GP, the main reason for the visit) as well as household characteristics (possession and use of computers), but not the demographic characteristics of respondents.

ASIs that describe the specific effects of addiction, as well as other characteristics that describe dependence (the length of taking drugs) had a predictive effect on the quality of life in the field of environment.

A higher level of quality of life in the domain of environment can be expected in patients with fewer consequences of addiction (lower ASI), longer period of being addicts, rarely interrupted participation in the methadone program, who normally ask doctor for help, live in urban areas, married or in a *de facto* relationship (Table 3).

Discussion

Although there is no statistically significant difference in the index of quality of life, the addicts in the methadone program perceive the quality of life significantly worse than the addicts treated with buprenorphine.

The differences produce a significantly greater participation of the categories of the respondents who perceive their quality of life as poor in the group A (addicts in the methadone program) and significantly greater participation of the categories of respondents who perceive the quality of life as very good in the group B (addicts of methadone program). It seems that the reasons for these differences lie not only in the characteristics of methadone treatment, bearing in mind that analysis has a predictive value of a large number of other characteristics that describe addicts and addiction.

Those who live in a household with more members and fewer children, have more education, and males in the city, in this study, perceive their quality of life better. The differences between addicts males and females can be explained in terms of gender specificity of men, already pointed to in other studies [2, 15]. Our findings also confirm observations about the importance of social networks [11, 15, 17] for the quality of life of drug users, as evidenced by the predictive value of a great households [14, 17]. In most households, there are all conditions for the significant support regarding instrumental (material, emotional) assistance, as well as conditions for the development of the sense of security (perception of support if needed). On the contrary, a proportional correlation between the number of children and the perception of quality of life can be explained by age (younger addicts have fewer children) and a few obligations towards children in the life of each man, even addicts. Other studies show similar results [1, 2].

A directly proportional correlation between health status (functional capacity indicated by the frequency of the problem in terms of mobility and the ability to care for themselves, the presence of limitations in their daily activities, often have signs of irritability and depression) and health perceptions, on one hand and the quality of life perception on the other, also supports the findings of other researchers regarding the connection between health and the quality of life [14, 15].

Closely related is the established importance of the consequences of addiction. The overall quality of life is perceived poorer by respondents who have higher ASI index (the presence of severe consequences of addiction). These results correlate with the findings of other researchers on the impact of consequences of addiction on the quality of life, which is realized directly [16] or in conjunction with other characteristics of the respondents [14, 15, 17].

The overall quality of life is perceived by better respondents involved in the methadone maintenance program, which confirms the findings of other researchers on the importance of long methadone treatment [3, 7–9, 11–14, 18, 20–27]. On the contrary, interruptions of treatment showed no predictive value.

The higher index of quality of life in the domain of physical health could be expected in patients with fewer expressed addicts consequences (lower ARI) and a shorter time length of being an addict, just as the other researchers claim [14, 15]. The results obtained in this study confirm the importance of functional capacity (lower frequency of problems in carrying out daily activities), better perception of health (higher grades of health), continuity of care (often paying visits to a doctor and regular health controls), which correlate with the findings of other researchers on determining the impact of symptoms and chronic problems [5, 14, 15] on the quality of life in the physical domain. The importance of living conditions, also emphasized by other researchers [14, 15, 18] confirmed the results of this research on determining the impact of housing conditions: addicts who live in smaller homes with central heating, reside in small towns, do physical work, have higher incomes, live in and out of wedlock, belong to categories with higher education – have a higher quality of life in the physical domain. Living conditions determine the gender-specific context [1, 2, 16] which can be explained by the predictive significance of gender, *ie*, a higher quality of life for male addicts. Characteristics of treatment are associated with an index of quality of life in the area of physical health.

A significant predictive effect on the index of quality of life regarding mental health has the length of drug abuse and the complex consequences of addiction, as well as the urban environment. A higher quality of life in this area could be expected in those with fewer expressed addicts' consequences (lower ASI), with fewer problems with taking care of themselves, fewer functional limitations (pain, discomfort), who perceive their own health better. The higher index of quality of life in the field of mental health have even those with longer period of being addicts, if they are more involved in the methadone program which they rarely abandon, and behave in a protective way (more often turn to doctor for help). This finding confirms the assumption about the length of the contribution and importance of the continuity of the methadone program, also claimed by the others [10, 20]. A higher quality of life in the psychological domain determines the number of features of urban social and economic position (higher education, higher income, occupation, life in the city, living in a home with larger area heated by solid fuel, a grea-

ter number of household members), as well as the (male) gender. Predictive effects of gender can be explained from a gender perspective: our findings confirm the observations on the impact of drugs, which is realized in cooperation with those of other (personal and environmental) characteristics (life situation, financial condition, changes in perspective, participation in leisure and social activities, support environment) that describe the position of male addicts in Serbian society [6].

Analysis of the predictors confirmed the predictive value of psychosomatic preservation of addicts and characteristics of family households in the quality of life in the domain of social relations. The addicts with better habitus (health status, and functional abilities) and less emphasized consequences of addiction (ASI of the consequences of addicts) have better social relationships with the environment. Characteristics of family households (households with more family members provide better social support) also contribute to it, which has already been discussed. Contrary to expectation, the characteristics of the treatment have no predictive effect on the index of quality of life in the domain of social relationships.

A higher level of quality of life in the domain of environment is determined by the characteristics of addiction (serious consequences of addiction, the length of drug abuse), but also treatment continuity (intermittent). These results correlate with the findings of other studies [7, 11]. Better relations in the region are typical for addicts who are referred to their environment (living in urban settings, in and out of wedlock), with developed patterns of protective behaviors (health check). Other researchers also indicate the importance of inclusion [2, 14, 22].

Conclusion

Treatment can improve the quality of life of opiate addicts, regardless of whether it is based on substitution of methadone or buprenorphine, but the effect of treatment is not equal in all the domains of quality of life.

Variations in the perception and the level of quality of life in different domains can not be explained by one predictor.

The number of the determined variables is large, and their action is complex.

The most common predictors of quality of life are health characteristics, the characteristics of the socioeconomic position of the respondents, as well as the various consequences of addiction.

The effect of treatment on the quality of life is less pronounced.

The highest predictive values among the characteristics are the duration of treatment and interruptions in methadone treatment. The overall quality of life is perceived better by respondents involved in the methadone maintenance program.

Future study on opiate addicts should be focused more on the quality of life assessed from the perspective of drug users.

REFERENCES

1. *Baharom N, Hassan MR, Ali N, Shah SA.* Improvement of quality of life following 6 months of methadone maintenance therapy in Malaysia. Subst Abuse Treat Prev Policy 2012; 7: 32.
2. *Feelemyer JP, Des Jarlais DC, Arasteh K, Phillips BW, Hagan H.* Changes in quality of life (WHOQOL-BREF) and addiction severity index (ASI) among participants in opioid substitution treatment (OST) in low and middle income countries: an international systematic review. Drug Alcohol Depend 2014; 134: 251−8.
3. *Maremmani I, Pani PP, Pacini M, Perugi G.* Substance use and quality of life over 12 months among buprenorphine maintenance-treated and methadone maintenance-treated heroin-addicted patients. J Subst Abuse Treat 2007; 33(1): 91−8.
4. *Xiao L, Wu Z, Luo W, Wei X.* Quality of life of outpatients in methadone maintenance treatment clinics. Acquir Immune Defic Syndr 2010; 53 Suppl 1: S116−20.
5. *Lashkaripour K, Bakhshani NM, Sadjadi SA.* Quality of life in patients on methadone maintenance treatment: A three-month assessment. J Pak Med Assoc 2012; 62(10): 1003−7.
6. *Padaiga Z, Subata E, Vanagas G.* Outpatient methadone maintenance treatment program. Quality of life and health of opioid-dependent persons in Lithuania. Medicina (Kaunas) 2007; 43(3): 235−41.
7. *Dhawan A, Chopra A.* Does buprenorphine maintenance improve the quality of life of opioid users. Indian J Med Res 2013; 137(1): 130−5.
8. *Chou YC, Shih SF, Tsai WD, Li CS, Xu K, Lee TS.* Improvement of quality of life in methadone treatment patients in northern Taiwan: A follow-up study. BMC Psychiatry 2013; 13: 190.
9. *Huong AG, Ng CG, Amer SA.* Quality of life assessment of opioid substance abusers on methadone maintenance therapy (MMT) in University Malaya Medical Centre. Asian J Psychiatr 2009; 10: 1–11.
10. *Parmenter J, Mitchell C, Keen J, Oliver P, Rowse G, Neligan I,* et al. Predicting biopsychosocial outcomes for heroin users in primary care treatment: a prospective longitudinal cohort study. Br J Gen Pract 2013; 63(612): e499−505.
11. *Pang L, Hao Y, Mi G, Wang C, Luo W, Rou K,* et al. Effectiveness of first eight methadone maintenance treatment clinics in China. AIDS 2007; 21 Suppl 8: S103−7.
12. *De Maeyer J, Vanderplasschen W, Camfield L, Vanheule S, Sabbe B, Broekaert E.* A good quality of life under the influence of methadone: A qualitative study among opiate-dependent individuals. Int J Nurs Stud 2011; 48(10): 1244−57.
13. *De Maeyer J, Vanderplasschen W, Lammertyn J, van Nieuwenhuizen C, Broekaert E.* Exploratory study on domain-specific determinants of opiate-dependent individuals' quality of life. Eur Addict Res 2011; 17(4): 198−210.
14. *De Maeyer J, Vanderplasschen W, Broekaert E.* Exploratory Study on Drug Users' Perspectives on Quality of Life: More than Health-Related Quality of Life. Soc Indic Res 2009; 90(1): 107−26.
15. *Marijanović M.* The influence of the length of methadone maintenance treatment on quality of life of opioid addicts. Racionalna terapija 2014; 6(1): doi: 10.5937/racter6-4300. (Serbian)
16. *Shekarchizadeh H, Ekhtiari H, Khami MR, Virtanen JI.* Patterns of pre-treatment drug abuse, drug treatment history and characteristics of addicts in methadone maintenance treatment in Iran. Harm Reduct J 2012; 9: 18.

17. *Rouhani S, Kheirkhah F, Salarieh I, Abedi S*. Quality of life, its early change and retention in MMT program in Iran: Evidence for policymakers and service providers. Life Sci J 2012; 9(3): 2633−8.
18. *Huissoud T, Rousson V, Dubois-Arber F*. Methadone treatments in a Swiss region, 2001-2008: A registry-based analysis. BMC Psychiatry 2012; 1 2: 238.
19. *De Maeyer J, Nieuwenhuizen C, Bongers IL, Broekaert E, Vanderplasschen W*. Profiles of quality of life in opiate-dependent individuals after starting methadone treatment: A latent class analysis. Int J Drug Policy 2013; 24(4): 342−50.
20. *Gossop M, Marsden J, Stewart D, Treacy S*. Outcomes after methadone maintenance and methadone reduction treatments: Two-year follow-up results from the National Treatment Outcome Research Study. Drug Alcohol Depend 2001; 62(3): 255−64.
21. *Tran BX, Ohinmaa A, Nguyen LT, Oosterhoff P, Vu PX, Vu TV*, et al. Gender differences in quality of life outcomes of HIV/AIDS treatment in the latent feminization of HIV epidemics in Vietnam. AIDS Care 2012; 24(10): 1187−96.
22. *Tran BX, Ohinmaa A, Nguyen LT, Nguyen TA, Nguyen TH*. Determinants of health-related quality of life in adults living with HIV in Vietnam. AIDS Care 2011; 23(10): 1236−45.
23. *Bawor M, Dennis BB, Anglin R, Steiner M, Thabane L, Samaan Z*. Sex differences in outcomes of methadone maintenance treatment for opioid addiction: A systematic review protocol. Syst Rev 2014; 16: 3−45.
24. *Millson P, Challacombe L, Villeneuve PJ, Strike CJ, Fischer B, Myers T*, et al. Determinants of Health Related Quality of Life (HRQOL) of Opiate Users at Entry to Low Threshold Methadone Programs. J Subst Use 2006; 11(2): 137−49.
25. *Tracy EM, Laudet AB, Min MO, Kim H, Brown S, Jun MK,* et al. Prospective patterns and correlates of quality of life among women in substance abuse treatment. Drug Alcohol Depend 2012; 124(3): 242−9.
26. *Zhao L, Holzemer WL, Johnson M, Tulsky JP, Rose CD*. HIV infection as a predictor of methadone maintenance outcomes in Chinese injection drug users. AIDS Care 2012; 24(2): 195−203.
27. *EuroQol Group*. EuroQol--a new facility for the measurement of health-related quality of life. Health Policy 199; 16(3): 199−208.
28. *Szende A, Oppe M, Devlin N*. EQ-5D value sets: Inventory, comparative review and user guide. Dordrecht: Springer; 2006.
29. *McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G,* et al. The Fifth Edition of the Addiction Severity Index. J Subst Abuse Treat 1992; 9(3): 199−213.
30. *World Health Organization*. Quality of life (WHOQOL-BREF). Procedures Manual. Geneva: World Health Organization; 2004.

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Osteoporosis-related knowledge among Serbian postmenopausal women

Znanje o osteoporozi kod žena u postmenopauzi u Srbiji

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Abstract

Background/Aim. Osteoporosis mainly affects women in the early years following menopause. The aim of this study was to determine the level of knowledge about osteoporosis and osteoporosis related risk factors in postmenopausal women in Serbia. **Methods.** The study included postmenopausal women regardless if suffering from osteoporosis or not. Assessment of knowledge was carried out by using the Osteoporosis Knowledge Assessment Tool - Shorter Version (OKAT-S) questionnaire that was validated for Serbian population. Answers to the 9 questions were coded as 1 –true, or 0 – false or "do not know". Also, the following risk factors data for osteoporosis were collected: age, the onset and duration of menopause, body mass index (BMI), data on fractures, the incidence of falls, smoking, lifestyle (active, sedentary), regular sunbathing, calcium and vitamin D supplementation, intake of milk and dairy products. **Results.** A total of 132 postmenopausal women responded to the questionnaire with the response rate of 90.41%. Their knowledge varied from 27.94% to 74.26% of the correct answers, with the average OKAT-S score of 4.5 (SD = 2.55), which was 50% of the maximum possible score. Only 2 participants (1.47%) filled the all OKAT-S items correctly, while 11 (8.09%) of them did not have the proper answer to any question. A reduced bone density (T-score below -1) was registered in 40.91% of the women, previous fractures in 49 (34.51%), and more or less 3 falls registered in 9.59% or 4.79%, respectively. **Conclusion.** The Serbian version of the questionnaire OKAT-S revealed generally poor knowledge on osteoporosis among postmenopausal women in Serbia. Developing effective interventions and public health programms could be helpful in general education towards understanding osteoporosis and risk factors. Promotion of preventive measures and healthy behaviour may prevent or at least slow down the accelerated bone loss in postmenopausal women.

Key words:
osteoporosis; osteoporosis, postmenopausal; risk factors; knowledge; attitude to health; serbia.

Apstrakt

Uvod/Cilj. Osteoporoza uglavnom pogađa žene u ranim godinama posle menopauze. Cilj rada bio je da se proceni znanje o osteoporozi i faktorima rizika kod žena u postmenopauzi u Srbiji. **Metode.** U studiju su bile uključene žene u postmenopauzi nezavisno od toga da li boluju od osteoporoze ili ne. Procena znanja o osteoporozi vršena je pomoću upitnika *Osteoporosis Knowledge Assessment Tool - Shorter Version* (OKAT-S) koji je validiran za srpsku populaciju. Odgovori na 9 pitanja kodirani su sa 1 – pravilan ili 0 – nepravilan odgovor ili „ne znam". Takođe, sakupljeni su sledeći podaci o faktorima rizika od osteoporoze: starost, početak i dužina trajanja menopauze, indeks telesne mase [*body mass index* (BMI)], podaci o prelomima, broj padova, pušenje, životni stil (sedeći ili aktivni), redovno sunčanje, dopuna kalcijumom i vitaminom D, korišćenje mleka i mlečnih proizvoda. **Rezultati.** Od ukupno 146 ispitanica, upitnik OKAT-S popunile su 132 ispitanice (nivo odgovora od 90,41%). Nivo njihovog znanja varirao je od 27,94% do 74,26% ispravnih odgovora, sa prosečnim OKAT-S skorom od 4,5 (SD = 2,55), što čini 50% od maksimalno mogućeg skora. Samo dve ispitanice (1,47%) pravilno su odgovorile na sva pitanja, dok njih 11 (8,09%) nije imalo nijedan ispravan odgovor. Snižen T-skor, manje od -1, registrovan je kod 40,91% ispitanica, a prethodni prelom kod 49 (34,51%). Kod 9,59% is-

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pitanica registrovano je više od 3, a kod 4,79% manje od 3 pada. **Zaključak.** Srpska verzija upitnika OKAT-S otkrila je generalno loše znanje o osteoporozi kod žena u postmenopauzi u Srbiji. Poboljšano znanje o osteoporozi i faktorima rizika moguće je postići razvojem efikasnih interventnih i javnih zdravstvenih programa. Promocija preventivnih mera i zdravog ponašanja može sprečiti ili bar usporiti prerani gubitak koštane mase kod žena u postmenopauzi.

Ključne reči:
osteoporoza; osteoporoza, posmenopauzalna; faktori rizika; znanje; stav prema zdravlju; srbija.

Introduction

Osteoporosis is the most common metabolic bone disease, and one of the main causes of mortality and morbidity in the elderly population. It is characterized by decreased bone mass and structural deterioration of bone tissue, leading to an increased bone fragility and susceptibility to fractures following minimal trauma [1].

Osteoporosis is a major cause of fracture in elderly, resulting in pain, disability, costly rehabilitation, poor quality of life, and premature death. Osteoporosis predominantly affects women who experience more rapid bone loss in the early years following menopause [2, 3].

Osteoporosis is a major and growing public health problem in developing countries. Knowledge of modifiable risk factors (smoking, lack of physical exercise, dietary habits) and treatment of osteoporosis should be targeted by prevention programmes. A key component in developing successful education intervention by health care professional is understanding what women know about the disease and to what extent they practice preventive behaviours [4].

The aim of the study was to assess the osteoporosis-related knowledge in Serbian postmenopausal women. Estimation of the level of knowledge of the population can help to guide public health programmes and plan education of patients in the field of osteoporosis.

The aim was also to assess the risk factors for osteoporosis as well as risk factors for osteoporotic fractures. According the above facts it is possible to successfully plan preventative measures, primarily by influencing on the modifiable-risk factors.

Methods

Patients/participants

During the meeting *"*Fair of the Third Age 55+*"*, held in Belgrade on 25–26 May 2015, free and voluntary peripheral bone mineral density (BMD) was measured on the heel, by the PIXY DXA (Dual X-Ray Apsoptiometry). Basic demographic data, previous fracture, non-modifiable and modifiable risk factors for osteoporosis were collected. All participants were asked to complete the questionnaire Osteoporosis Knowledge Assessment Tool - Shorter Version (OKAT-S), regardless of whether or not they have osteoporosis. Out of 146 scanned patients, 132 (90.41%) filled the questionnaire.

The questionnaire OKAT-S

The OKAT-S covers knowledge on preventive behaviour, risk factors, and consequences of osteoporosis. It is a 9-item questionnaire with three possible answers on every item: true, false, and do not know (correct answers were scored as 1, an incorrect or "do not know" as 0). The final score presented the sum of all correct answers and could be in range from 0 to 9 (with the higher scores indicating better knowledge). The questionnaire OKAT-S, presented good psychometric characteristics, was validated for the Serbian population and therefore could be used for knowledge assessment as single underlying factor [5].

Statistical analysis

The data were analyzed using the descriptive statistics measures (mean values, standard deviations and frequencies). Internal consistency of the questionnaire was tested using Cronbach's coefficient.

Results

The study included 132 postmenopausal women. The most of them had no osteoporosis or osteopenia. Their characteristics are presented in Table 1.

Osteoporosis screening showed that 78 (59.09%) participants had normal BMD, whereas 54 (40.91%) had osteopenia or osteoporosis. About a third of the patients had a fracture (n = 49, 34.51%). The average number of fractures was 1.47 (SD = 0.95) in all the patients. The most frequent fracture sites were arm (n = 14, 9.59%), and leg fractures (n = 10, 6.85%). There was only one patient with hip fracture and five with vertebral fracture. Only two patients had the history of their parents' hip fracture.

Exactly 9.59% of the patients experienced fall more than three times and 4.79% less than three times. Falls occurred more outdoor (10.27%) than inside the house (4.79%).

From all the patients, 132 answered the questionnaire OKAT-S with a response rate of 93.15%. This questionnaire presented good internal consistency (Cranach's $\alpha = 0.70$).

Table 2 presents the results from the OKAT-S with the percentage of correct answers for each item. Only four items were anwered correctly by more than 50% of the respondents. The most frequent correct answer was about occurrence of osteoporosis according to the gender and the most incorrect answer was about item "there is a small amount of bone loss in the 10-year following the onset of menopause". The levels of knowledge varied between 27.94% (Q8) and 74.26% (Q2).

The mean score was 4.5 (SD = 2.25, range of correct answers 0-9), out of a possible 9 points on the OKAT-S, being 50% of possible maximum score. Only 2 (1.47%) patients filled the all OKAT-S items correctly, while 11 (8.09%) did not have any correct answer (Figure 1).

Table 1

Characteristics of the participants

Variables	Postmenopausal women (n = 132)
Non-modifiable risk factors	
age (years), $\bar{x} \pm SD$	65.94 ± 8.04
menopause (missing n = 12), n (%)	120 (90.9)
age when menopause started (years), $\bar{x} \pm SD$	48.97 ± 4.78
duration of menopause (years), $\bar{x} \pm SD$	9.38 ± 8.02
height (cm), $\bar{x} \pm SD$	163.48 ± 6.86
Peripheral BMD > 1	78 (59.09%)
T-score < 1	54 (40.91%)
Modifiable risk factors	
weight (kg), $\bar{x} \pm SD$	70.00 ± 11.61
BMI (kg/m^2), mean value (SD)	26.14 ± 3.74
BMI categories, n (%)	
underweight	3 (2.27)
normal	37 (28.03)
pre-obese	75 (56.82)
obese	17 (12.88)
smoking, n (%)	
current smoker	17 (12.88)
ex smoker	9 (6.82)
no	106 (80.30)
lifestyle (missing n = 5), n (%)	
active	103 (81.10)
sedentary	24 (18.89)
sunbathing for 15 days a year (missing n = 1), n (%)	82 (62.59)
vitamin D supplementation (missing n = 1), n (%)	33 (25.19)
calcium supplementation (missing n = 2), n (%)	29 (22.31)
milk products intake (missing n = 2), n (%)	91 (70.00)

BMI – body mass index; BMD – bone mineral density; SD – standard deviation.

Table 2

Knowledge about osteoporosis – analyse results of Osteoporosis Knowledge Assesment Tool - Shorter Version (OKAT-S) questionnaire in postmenopausal women in Serbia

Questions		Correct answer	Correct answers n (%)
Q1	Osteoporosis usually causes symptoms (e.g., pain) before fractures occur	False	45 (33.09)
Q2	Osteoporosis is more common in men	False	101 (74.26)
Q3	Cigarette smoking can contribute to osteoporosis	True	95 (69.85)
Q4	White women are at highest risk of fracture as compared to other races	True	59 (43.38)
Q5	By age 80, the majority of women have osteoporosis	True	84 (71.76)
Q6	Family history of osteoporosis strongly predisposes a person to osteoporosis	True	91 (66.91)
Q7	Alcohol in moderation has little effect on osteoporosis	True	44 (32.35)
Q8	There is a small amount of bone loss in the 10 years following the onset of menopause	False	38 (27.94)
Q9	Hormone therapy prevents further bone loss at any age after menopause	True	55 (40.44)

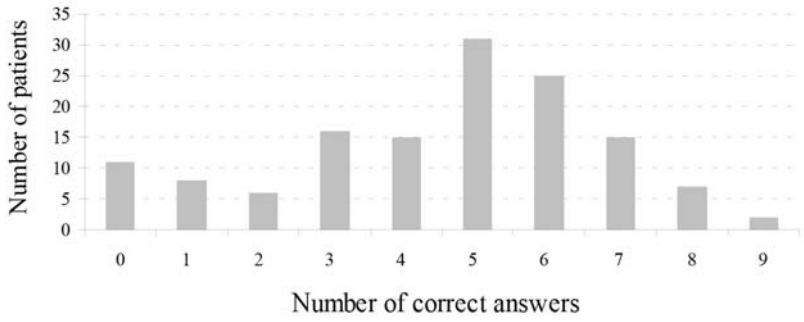

Fig. 1 – Osteoporosis Knowledge Assessment Tool – Shorter Version (OKAT-S) scores (sum of correct answers) in relation to the number of the patients.

Discussion

Study subjects were surveyed using a valid and reliable questionnaire OKAT with good psychometric properties [5, 6]. OKAT covers the core knowledge about osteoporosis and is very useful as a baseline, as well as follow-up measurement when implementing educational interventions.

The tool had questions that assess four basic themes: knowledge of the symptoms understanding (symptoms and risk of fracture), the knowledge of risk factors for osteoporosis, knowledge of preventive factors suh as physical activity and diet relating to osteoporosis, and treatment availability.

Numerous studies have found serious lacks of knowledge about osteoporosis and related risk factors. A worrying deficit of knowledge with 39.6% of possible maximum score on the OKAT was found in Syrian young adult females students at nursing school in Damascus [7]. A sample of 250 female students studying at the Faculty of Pharmacy at the University of Belgrade revealed also poor knowledge about osteoporosis with 41.55% of possible maximum OKAT score [5]. Baseline levels of osteoporosis knowledge measured using the OKAT were low in Australian women where the tool was origialy implemented. The avarege score was 8.8 out of 20, being 44% of the possible maximum score. The average age of Australian participants was 37.8 years [6].

In fact, without adequate knowledge women will not be able to determine their own risk of developing the disease and changing to health related behaviours. In spite of the fact that the vast majority of respondents in this study were able to identify osteoporosis as an elderly women disorder (Q5, 71.76%), with strong genetic influence (Q6, 66.91%) the most of them did not recognize the nature of osteoporosis. Since osteoporosis is a "silent disease", most people are not aware of their condition until they experience a fragility fracture. Only a smaller percentage (Q1, 33%) our participants recognized the "silent" nature of the disease. Although this response is unsatisfactory, other authors found a much lower percentage (9.1%) of correct answers to the question Q1. The mean score in Arabic population reffering to knowledge of less than 50% of the OKAT questions [8]. Furthermore, our study showed very poor knowledge about the identification of post-menopausal conditions as a period of accelerated bone loss (Q8, 27.94), so as that this bone loss can be prevented by the application of hormone therapy (Q9, 40.44%).

Our investigation showed that multiple risk factors for osteoporosis and fractures were present. Some of the risk factors are unchangeable, such as the Caucasian race, female gender, advanced age, postmenopausal status, as well as prolonged menopause (over 9 years of duration). We revealed a very low percentage of decreased BMI (below 3%) which could be protective factor for osteoporosis. Only 1/4 of postmenopausal women received calcium and vitamin D supplementation, 40% were not regularly exposed to the sun, around 20% had sedentary, non-active lifestyle, and about 13% of them were current smokers. These are the areas which should focus on educating the population and preventive measures. Furthermore, screening dual energy x-ray absorptiometry (DXA) measurements in this study showed a reduced BMD in 40% of the sample, which requires further diagnostic procedures such as bone scan at the hip and spine by a central DXA. About 1/3 of all postmenopausal women had previous osteoporotic fracture. All the experts agree on the fact that there is evidence that previous osteoporosis-related fractures in both men and women comprise a risk factor for new fractures [1–3].

Some studies have revealed that education programmes for the elderly are effective in improving health promotion knowledge and behaviours [9]. Previous studies have shown positive results in patient education to improve the use of non-pharmacologic preventive measures for osteoporosis [10, 11]. Knowledge of modifiable risk factors (smoking, lack of physical exercise, dietary habits) should be targed by prevention programmes. Estimation of the level of knowledge in the population can help to guide public health programmes [4].

Osteoporosis is not curable, but it can be prevented in part by incrasing the level of physical activity at all ages, cessation of smoking, reduction of alcohol consumption, adequate calcium and vitamin D intake, and fall prevention [1].

The current study has a number of potential limitations. It used a relatively small sample and the study was based on urban retired postmenopausal women, so the findings cannot be generalized to the entire postmenopausal women in Serbia.

Conclusion

The Serbian version of the questionnaire OKAT-S revealed generally poor knowledge on osteoporosis among postmenopausal women in Serbia. Developing effective interventions and public health programms could be helpful in general education towards understanding osteoporosis and risk factors. Promotion of preventive measures and healthy behaviour may prevent or at least slow down the accelerated bone loss in postmenopausal women.

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R E F E R E N C E S

1. *Kanis JA*.World Health Organization Scientific Group. Assessment of osteoporosis at the primary health care level Technical Report. Sheffield, United Kingdom: World Health Organization Collaborating Centre for Metabolic Bone Diseases; 2007.
2. National Osteoporosis Foundation. Clinician's Guide to Prevention and Treatment of Osteoporosis. Washington, DC: National Osteoporosis Foundation; 2010.
3. Scottish Intercollegiate Guidelines Network. SIGN 71. Management of osteoporosis. Edinburgh: SIGN; 2003.
4. *Riaz M, Abid N, Patel J, Tariq M, Khan MS, Zuberi L*. Knowledge about osteoporosis among healthy women attending a tertiary care hospital. J Pak Med Assoc 2008; 58(4): 190−4.
5. *Tadic I, Stevanovic D, Tasic L, Vujasinovic Stupar N*. Development of a shorter version of the osteoporosis knowledge assessment tool. Women Health 2012; 52(1): 18−31.
6. *Winzenberg TM, Oldenburg B, Frendin S, Jones G*. The design of a valid and reliable questionnaire to measure osteoporosis knowledge in women: The Osteoporosis Knowledge Assessment Tool (OKAT). BMC Musculoskel Dis 2003; 4(1): 17.
7. *Sayed-Hassan R, Bashour H, Koudsi A*. Osteoporosis knowledge and attitudes: across-sectional study among female nursing school students in Damascus. Arch Osteoporos 2013; 8: 149.
8. *Sayed-Hassan RM, Bashour HN*. The reliability of the Arabic version of osteoporosis knowledge assessment tool (OKAT) and the osteoporosis health belief scale (OHBS).BMC Res Notes 2013; (6): 138.
9. *Huang L, Chen S, Yu Y, Chen P, Lin Y*. The effectiveness of health promotion education programs for community elderly. J Nurs Res 2002; 10(4): 261−70.
10. *Bångsbo A, Björklund A*. Professional views on patient education in osteoporosis. Arch Osteoporos 2010; 5(1): 101−10.
11. *Levinson MR, Leeuwrik T, Oldroyd JC, Staples M*. A cohort study of osteoporosis health knowledge and medication use in older adults with minimal trauma fracture. Arch Osteoporos 2012; 7(1−2): 87−92.

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Total hip arthroplasty for femoral neck fractures as an urgent procedure

Totalna artroplastika kuka za prelome vrata femura kao urgentna procedura

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Abstract

Background/Aim. Total hip arthroplasty (THA) is one of the most widely accepted operative methods for femoral neck fracture (FNF) in elderly. However, the data on the early THA for FNF are very limited. The aim of this study to determine if there were differences in postoperative complications and functional outcomes between an urgent and delayed THA following FNF. **Methods**. This prospective study included a total of 244 patients who had THA following FNF from January 2010 to January 2013. In the first group 41 FNF patients were treated with THA within less than 12 hours of admission. A total of 203 FNF patients were operated in delayed settings, of whom 162 required prolonged preoperative processing and comorbidities correction. The group II consisted of 41 FNF patients who were fit for the early surgery at admission, but the operation was delayed due to institution related reasons. Main outcome measurements included mortality, functional outcome assessement, cardiological and pulmonary complications, pressure ulcers, dislocations, infections, length of hospitalization and revisions. **Results**. There were no differences in terms of age, gender, type of implants, neither in mortality, nor complications. There were differences in hospital length of stay [t (51.72) = -10.25, $p < 0.001$)]. The patients operated within less than 12 hours of admission, had significantly better scores at all three time points of functional outcome assessment: at discharge t (80) = 2.556, $p < 0.012$; one month t (80) = 4.731, $p < 0.001$; three months t (80) = 5.908, $p < 0.001$. **Conclusion**. THA for FNF as an urgent procedure is not a widely accepted concept. Our findings indicate that the early operative treatment, does not worsen clinical outcomes, and our results give an advantage to the policy of the early THA for FNF.

Key words:
femoral neck fractures; arthroplasty, replacement, hip; time factors; treatment outcome; orthopedic procedures.

Apstrakt

Uvod/Cilj. Totalna artroplastika kuka (*total hip arthroplasty* – THA) je jedna od najčešće primenjivanih metoda za lečenje preloma vrata femura (*femoral neck fracture* – FNF) kod starijih. Međutim, retki su podaci o ranoj primeni THA za FNF. Cilj ovog rada bio je da se utvrdi razlika u postoperativnim komplikacijama i funkcionalnom ishodu između urgentne i odložene THA nakon FNF. **Metode**. Ovom prospektivnom studijom obuhvaćena su 244 bolesnika kojima je izvedena THA nakon FNF u periodu od januara 2010. do januara 2013. U prvoj grupi, kod 41 bolesnika izvedena je THA u prvih 12 sati od prijema. Ukupno 203 bolesnika su operisana odloženo, od kojih je kod 162 bila potrebna dodatna korekcija komorbiditeta. Zbog toga je druga grupa sastavljena od bolesnika (n = 41) koji su mogli da budu operisani neposredno po prijemu, ali je operacija odlagana usled institucionalnih razloga. Praćeni su mortalitet, funkcionalni ishod, kardiološke i pulmološke komplikacije, dekubitusi, dislokacije, infekcije, dužina hospitalizacije i revizije. **Rezultati**. Nije bilo razlika u godinama starosti, polu, tipu implantata, mortalitetu niti komplikacijama. Utvrđene su razlike u dužini bolničkog lečenja [t (51.72) = -10.25, $p < 0.001$]. Bolesnici operisani u prvih 12 časova od prijema imali su značajno bolje skorove u sva tri vremena procene funkcionalnog ishoda: na otpustu, t (80) = 2.556, $p < 0.012$; nakon jednog meseca, (80) = 4.731, $p < 0.001$; i nakon tri meseca, t (80) = 5.908, $p < 0.001$. **Zaključak**. Metoda THA kao urgentna procedura za FNFs nije široko prihvaćen koncept. Naši nalazi pokazuju da rani operativni tretman ne pogoršava klinički ishod. Rezultati daju jasnu prednost ranoj THA kod bolesnika sa FNF.

Ključne reči:
femur, prelomi vrata; artroplastika kuka; vreme, faktor; lečenje, ishod; ortopedske procedure.

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Introduction

Femoral neck fractures (FNF) as well as hip fractures in general, represent a major challenge for today's healthcare systems, mostly due to mortality, morbidity, and frequent lengthy hospitalization followed by a number of complications [1, 2].

Patients with hip fractures are faced with significantly higher rates of mortality and morbidity than those patients of the same age without fractures [3]. Mortality rates within a year of FNF range from 12% to 36% [4].

Despite the fact that 50% of the total hip fracture population has a displaced FNF, the optimal surgical option for FNF in the elderly is still the subject of an ongoing scientific and clinical debate [5, 6].

Traditionally, hip hemiarthroplasty (HA) and total hip arthroplasty (THA) are the most widely accepted operative methods for FNF in the elderly. Taking all the advantages into account (reduced dislocation rates, simple procedure, shorter operating time, less blood loss, lower initial costs), a number of authors prefer HA to THA [7, 8]. On the other hand, there is ample evidence that THA, with its advances in operative techniques and implants, provides better function, satisfaction, offers fewer complications and reduces the need for revisions [9–11].

In addition, there is an ongoing controversy regarding the ideal timing of operative treatment [12, 13]. Numerous studies have linked the increase in mortality and complication rates with delay in surgical treatment [14–17].

Although the impact of surgical delay on the early mortality after hip fractures has been extensively presented in the orthopaedic literature, the data on the subject of the early THA for FNF are exceptionally limited.

Therefore, the aim of this study was to determine if THA, performed as an urgent intervention for FNF, within 12 hours of admission, affects the outcome in relation to the same but delayed procedure.

Methods

We conducted a prospective comparative study in a single center, university hospital, to determine differences if any in postoperative complications and functional outcomes between an urgent and delayed THA following FNF. The study included a total of 244 patients who had THA following FNF from January 2010 to January 2013.

The inclusion criteria for THA after FNF were as fallows: FNF patients, aged 60 to 80 years, without mental and neurological impairments, the American Society of Anesthesiologists (ASA) score grade I/II. Additional prerequisites were that patients sustained a FNF on the day of admission, and that they were fit for the early surgery, with no need for preoperative comorbidities correction.

The follow-up protocol consisted of physical and radiological assessments, and those were done at discharge, one month, 3 months, 1 and 2 years after the surgery. The patients were regularly postoperatively followed-up for at least 2 years (average 2.6 years). All the patients were available for the follow-ups.

In the group I, 41 FNF patients were treated with THA within less than 12 hours of admission. A total of 203 FNF patients were operated in delayed settings, of whom 162 required prolonged preoperative processing and comorbidities correction. Therefore, the group II consisted of 41 FNF patients who were fit for the early surgery at admission, but the operation was delayed due to institution related reasons (weekends, holidays, unavailability of operating theaters or surgeons who routinely perform THA).

Preoperatively, all the patients were evaluated by anesthesiologists and if needed by other medical specialists. Most commonly requested consultations were by cardiologists, endocrinologist and pulmonologists. Preoperative evaluations of FNF patients, besides orthopedic examinations, routinely included complete blood counts, hematocrit and hemoglobin levels, biochemical analyses, electrolyte levels, EKG and chest radiograph, as well as some additional examinations requested by other specialists.

All the patients in both series received intravenous antibiotic prophylaxis consisting of the second- or third-generation cephalosporins, and low-molecular-weight heparins (LMWH) administered for 4 weeks postoperatively. The patients in the delayed group were given thromboprophylaxis preoperatively. General anesthesia was performed in all the cases. Surgical approach was posterolateral. Immediate weight bearing was allowed in all cases, and physical therapy was started on the first postoperative day. The time of admission was registered as the time when a patient arrived at the emergency room (ER). The patients in the early surgery group were transferred to the ward after operation.

Outcome assessments included mortality, pressure ulcers, cardiac complications, functional outcome assessment by the Harris hip score (HHS), dislocations, infections, length of hospitalization and revisions.

As far as THA implants are concerned, 28 mm heads (metal/polyethylene combination) were used in both investigated groups (Figures 1 and 2).

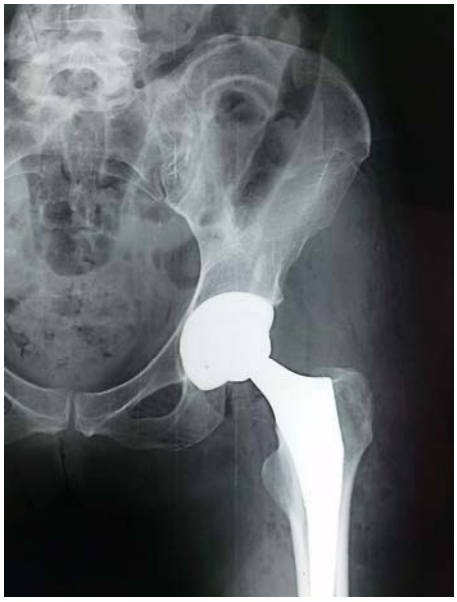

Fig. 1 – Cementless total hip arthroplasty.

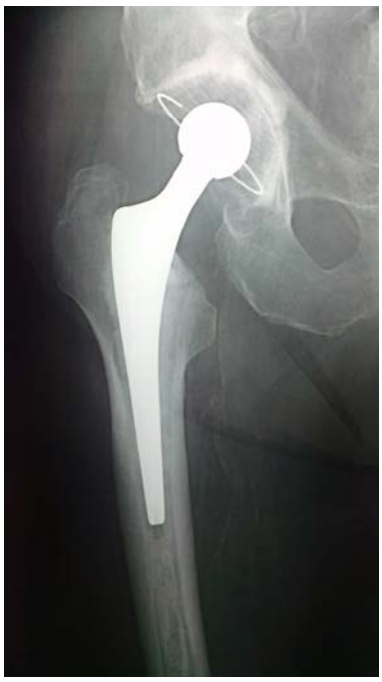

Fig. 2 – Cemented total hip arthroplasty.

Statistical analysis

Statistical analysis was performed using statistical package PASW 18. Long rank analysis of the null hypotesis was performed between the two timing approaches (with $p < 0.05$).

All procedures performed in the study were in accordance with the ethical standards of the institution and/or the National Research Committee, as well as with the 1964 Declaration of Helsinki and its later amendments. Moreover, informed consent was obtained from all individual participants included in the study.

Results

In the group I operated in the period less than 12 h of admission there were 31 females and 10 males, and the average age was 66.8 years (range 60–81). According to the American Society of Anesthesiologists (ASA), 24 patients were in ASA risk group 1, and 17 patients were in the ASA risk group 2. In the group II (operated on in delayed settings) there were 32 females and 9 males, and the average age was 67.5 years (range 62–78); 26 patients were in ASA risk group 1, and 15 were in the ASA risk group 2. Comorbidities, risk factors and types of THA used in both groups of patients are presented in Table 1.

According to the interval from admission to the surgery, in the group I there were 12 patients in 0–6 hours period in whom THA was performed.

In the group II THA was performed in the following way: in a 12–24 h post admission period in one patient; 24–48 h in 8 patients; 2–4 days in 18 patients; 5–6 days in 12 patients and 7–8 days in 2 patients.

Most of the patients in the group I were operated on during the late afternoon, evening and early morning hours. In the early group we performed 18 THAs, in the period from 3 pm to 10 pm, and 23 THAs were performed during the night in the period from 10 pm to 5 am.

One patient (a 67-year-old woman) in the delayed group died at her home 6 days after her discharge from hospital, on the 12th postoperative day. The autopsy report showed that pulmonary embolism and cardiac arrest were the cause of her death. The hybrid THA was performed in that case.

A patient (81-year-old male, cemented THA) in the delayed group, and a patient (76-year-old woman) in the group I died within the first year of the surgery (8 and 11 months postoperatively, respectively), due to the reasons that can not be directly linked with their hip fractures or consequent THA surgeries. During the follow-up period neither of the patients in both groups showed signs of aseptic loosening of any prosthetic component. Two patients with THA infections and septic loosening required revision procedures, so the two-stage revision with articulating hip spacers was performed in both cases.

Both groups had the same number of male and female patients ($\chi^2(1) = 0.069$, $p < 0.794$), as well as the same number of cementless, cemented and hybrid THAs ($\chi^2(2) = 0.271$, $p < 0.873$). *T*-test analysis revealed no difference between the two groups when it comes to the age of patients [$t\,(80) = 0.587$; $p < 0.559$].

Table 1

Comorbidities, risk factors and type of total hip arthoplasty (THA) according to the timing of surgery

Parameter	Group I	Group II	χ^2	*p* - values
Hypertension	9	8	$\chi^2(1) = 0.074$	< 0.785
Well controlled diabetes type 2	3	2	$\chi^2(1) = 0.213$	< 0.644
Stable angina	1	1	-	-
Rheumatoid arthritis	1	1	-	-
Well controlled thyroid disorder	0	1	$\chi^2(1) = 1.012$	< 0.314
Obesity	2	3	$\chi^2(1) = 0.213$	< 0.644
Smoking	2	2	-	-
Alcoholism	0	1	$\chi^2(1) = 1.012$	< 0.314
Uncemented THA	27	29	$\chi^2(1) = 0.225$	< 0.635
Cemented THA	11	9	$\chi^2(1) = 0.265$	< 0.607
Hybrid THA	3	3	-	-

Group I – THA performed in the period less than 12 hours of admission;
Group II – THA performed in delayed settings.

The average length of hospital stay in the group I was 7.1 (range 5 to 9) days, and in the group II it was 13.5 (range 7 to 19) days. The data relate to the initial hospitalizations after fractures, not to the days of revisions. The patients in the delayed group had the mean postoperative stay of 8.6 days. The patients who were operated within less than 12 hours required shorter hospitalization ($\bar{x} \pm SD = 7.1 \pm 1.446$ days) than it was the case with these operated on in the delayed procedure ($\bar{x} \pm SD = 13.5 \pm 3.736$ days). *T*-test revealed a statistical significance between the groups, $t\ (51.72) = -10.25$, $p < 0.001$. In comparison of just postoperative length of stay (eliminating the amount of time the patient spent in hospital prior to surgery) the value of t-test was $t\ (69.97) = -3.79$, $p < 0.001$.

The patients operated within less than 12 hours of admission, had significantly better scores at all three time points of functional outcome assessment: at discharge $t\ (80) = 2.556$, $p < 0.012$; one month $t\ (80) = 4.731$, $p < .001$; three months $t\ (80) = 5.908$, $p < 0.001$.

There would not be any statistically significant differences regarding functional outcome at the time point of three months ($t\ (80) = 1.936$, $p < 0.058$) if patients in the delayed group who needed revision procedures, were excluded from the final functional outcome assessment.

When it comes to postoperative complications, even though there were more complications in the group operated in the delayed procedure, those differences were not statistically significant. All registered dislocations in both groups were one-time events, noted within the first 6 postoperative weeks and did not require surgical revisions. The results of χ^2-tests are shown in Table 2. Log rank analysis of the null hypothesis was performed (Mantel $\chi^2 = 1.00$ $p < 0.317$) and there were no significant differences in mortality between these two timing approaches.

surgical techniques, and choice of implants, as well as influence of comorbidities, and postoperative care modalities that vary across different institutions and countries, so as to hinder the unification and standardization of optimal treatment.

There is limited research on timing of THA for FNF, and even less data are available on the subject of the early THA for FNF. A recent study on the early hip fracture surgery [18] finds that surgeries done within 36 hours of FNF were associated with reduced mortality compared to the surgeries done after that time. The same outcomes were shown for the surgeries done within 12, 24, 48, 60 and 72 hours. The authors have not found the minimum amount of time prior to which surgery failed to confer a survival benefit, but the limiting factor for comparison with our analysis, was that only one THA procedure (out of 2,056 patients) was registered. According to one systematic review and meta-analysis [17], early surgery was associated with a lower risk of death and lower rates of postoperative pneumonia and pressure sores among elderly patients with hip fractures. Rudelli et al. [11], who strongly advocate THA for FNF, performed 88 THAs over the period of 12 years for FNF, and reported time frame of 8 hours to 34 days, from fracture to operation, with patients in ASA groups 1 to 4 and hospitalization range of 6 to 35 days. But they have not clearly defined, neither have they studied differences between patients operated early and those THAs performed in delayed settings.

Several authors noted a higher incidence of complications after THA for FNF: Sharma et al. [19] reported 22% of local complications and 19% of general complications (7% of deep vein thrombosis), Narayan et al. [20] found 4% of dislocations, Džupa et al. [21] reported 9% of revisions.

Jameson et al. [22] analyzed the UK National Joint registry THA data for acute FNF (4323 procedures were studied) and the following was found: 3.25% of revision rate,

Table 2

Postoperative complications and functional outcome according to Harris hip scores (HHS)

Parameter	Group I	Group II	χ^2 / *t*	*p* - value
Infection	0	3	$\chi^2(1) = 3.114$	< 0.078
DVT	0	3	$\chi^2(1) = 3.114$	< 0.078
PE	0	3	$\chi^2(1) = 3.114$	< 0.078
Cardiologic complications	1	4	$\chi^2(1) = 1.917$	< 0.166
Pressure ulcers	0	2	$\chi^2(1) = 2.050$	< 0.152
Dislocations	2	3	$\chi^2(1) = 0.213$	< 0.644
Revisions	0	2	$\chi^2(1) = 2.050$	< 0.152
HHS at discharge	59.4	51.6	$t\ (80) = 2.556$	< 0.012
HHS 1 month after surgery	87.3	75.3	$t\ (80) = 4.731$	< 0.001
HHS 3 months after surgery	95.3	89.2	$t\ (80) = 5.908$	< 0.001

Group I – total hip arthroplasty (THA) performed in the period less than 12 hours of admission; Group II – THA performed in delay settings; DVT – deep venous thrombosis; PE – pulmonary embolism.

Disscusion

The impact of surgical delay on the outcome following hip fractures has been extensively presented in the orthopedic literature. When it comes to the proximal femur fractures research, there are other major concerns besides the timing of surgery, such as significant diversity of fracture patterns,

3.2% of mortality within 90 days and increased risk of mortality with higher ASA score. Thus, they concluded that there are no benefits of using head sizes > 28 mm or bearings other than metal-on-polyethylene, and that THA is a good option in patients aged < 75 years and with ASA 1/2.

Chaudhry et al. [23] reviewed the randomized controlled trials involving THA approach for FNF. Even though their

study did not evaluate the timing of surgery, they identified considerable variability in both the surgical approach and choice of implants. Therefore, they determined that standardization was needed in order to reduce complication rates.

FNF mortality rates, for arthroplastic procedures, range from 4.3% to 20% in the first 4 months following the surgery, and from 4.3% to 48% in the first postoperative year [24]. Specifically for FNF, albeit disregarding the influence of timing, Sebestyén et al. [25] concluded that gender, age and accompanying diseases significantly influence early mortality, whereas early postoperative complications do not have a significant impact on the mortality risk. Despite the fact that patients in both our series were older than 60 years and had hip fractures, early and late mortality rates were much lower (5% to 11.7%) than in other reports [26, 27]. It is likely due to the study inclusion criteria. One of the major concerns of THA for FNF lies in the possible higher incidence of dislocations, various authors reported different rates, ranging from 0% to 22% [24, 28]. The incidence we reported in both groups, was in the typical range reported by other authors. There were no statistically significant differences in procedure-related specific complications, such as dislocations and infections, even though 69% of THA in the 12-hour group were performed during the evening and night hours.

Low mortality rates as well as other complications registered, which are generally atypical of hip fractures, could be explained by patient selection and low ASA scores. Since the patients in both groups did not require prolonged comorbidities correction, burden of comorbidities which is typical for hip fractures, did not influence the delay. We may assume that differences in functional outcome and hospital stay were not caused by the presence or absence of comorbidities, but by the delay itself.

In line with previous reports, we found direct association between surgical delay and length of hospital stay and functional outcome. There was no significant statistical difference in mortality and complications between the two groups, but when it comes to mortality, the inspection of survival function gives a slight advantage to the group of patients who were operated on within less than 12 hours of admission, and the difference in infections, deep vein thrombosis and pulmonary embolism was on the verge of statistical significance in favor of early surgery.

Limitations

The number of patients enrolled in this study is lower than in other papers dealing with hip fractures. Therefore, it limits the accuracy and statistical power of the study. Narrowing the criteria for enrollment enabled the homogeneity but also reduced the number of patients in both groups. Power analysis revealed that sample sizes of both groups should have been larger (317 patients in each group) at the given probability of type I error of 0.05 and probability of type II error of 0.2 for the recorded mortality rate of 2.44%, to show a statistically significant difference between the two groups. Even though the study might be underpowered to demonstrate a significant difference in mortality and complication rates, it shows that early surgery may have a positive effect on functional outcome and length of hospital stay.

Conclusion

THA for FNF as an urgently performed procedure is not a widely accepted concept. The literature on the subject is consequently insufficient and without clear evidence to support or challenge the fast track approach.

Our findings indicate that immediate operative treatment, THA for FNF, is not associated with worse outcomes, and our results give an advantage to the policy of early THA for FNF in ASA 1 and 2 patients. Standardization efforts towards optimal outcomes of FNF, besides pre- and postoperative management, surgical approach and choice of implants, should consider the concept of early surgery in appropriately selected THA for FNF cases.

Conflict of interest statement

Each author certifies that he has no commercial associations (e.g., consultancies, stockownership, equity interest, patent/licensingarrangements, etc.) that might pose the conflict of interest regarding the submitted article.

REFERENCES

1. *Bhandari M, Devereaux PJ, Tornetta P 3rd, Swiontkowski MF, Berry DJ,Haidukewych G,* et al. Operative management of displaced femoral neck fractures in elderly patients. An international survey. J Bone Joint Surg Am 2005; 87(9): 2122−30.
2. *Johnell O, Kanis JA.* An estimate of the worldwide prevalence, mortality and disability associated with hip fracture. Osteoporos Int 2004; 15(11): 897−902.
3. *Hagino H, Nakamura T, Fujiwara S, Oeki M, Okano T, Teshima R.* Sequential change in quality of life for patients with incident clinical fractures: a prospective study. Osteoporos Int 2009; 20(5): 695−702.
4. *Miyamoto RG, Kaplan KM, Levine BR, Egol KA, Zuckerman JD.* Surgical management of hip fractures: an evidence-based review of the literature. I: femoral neck fractures. J Am Acad Orthop Surg 2008; 16(10): 596–607.
5. *Parker MJ, Gurusamy KS, Azegami S.* Arthroplasties (with and without bone cement) for proximal femoral fractures in adults. Cochrane Database Syst Rev 2010; (6): CD001706.
6. *Burgers PT, Van Geene AR, Van den Bekerom MP, Van Lieshout EM, Blom B, Aleem IS,* et al. Total hip arthroplasty versus hemiarthroplasty for displaced femoral neck fractures in the healthy elderly: a meta-analysis and systematic review of randomized trials. Int Orthop 2012; 36(8): 1549−60.
7. *Tanous T, Stephenson KW, Grecula MJ.* Hip hemiarthroplasty after displaced femoral neck fracture: a survivorship analysis. Orthopedics 2010; 33(6): 385.
8. *van den Bekerom MP, Hilverdink EF, Sierevelt IN, Reuling EM, Schnater JM, Bonke H,* et al. A comparison of hemiarthroplasty with total hip replacement for displaced intracapsular fracture of the femoral neck: a randomised controlled multicentre trial

in patients aged 70 years and over. J Bone Joint Surg Br 2010; 92(10): 1422−8.
9. *Cho MR, Lee HS, Lee SW, Choi CH, Kim SK, Ko SB.* Results after total hip arthroplasty with a large head and bipolar arthroplasty in patients with displaced femoral neck fractures. J Arthroplasty 2011; 26(6): 893−6.
10. *Schmidt AH, Leighton R, Parvizi J, Sems A, Berry DJ.* Optimal arthroplasty for femoral neck fractures: is total hip arthroplasty the answer? J Orthop Trauma 2009; 23(6): 428−33.
11. *Rudelli S, Viriato SP, Meireles TL, Frederico TN.* Treatment of displaced neck fractures of the femur with total hip arthroplasty. J Arthroplasty 2012; 27(2): 246−52.
12. *Flierl MA, Gerhardt DC, Hak DJ, Morgan SJ, Stahel PF.* Key issues and controversies in the acute management of hip fractures. Orthopedics 2010; 33(2): 102−10.
13. *Khan SK, Kalra S, Khanna A, Thiruvengada MM, Parker MJ.* Timing of surgery for hip fractures: a systematic review of 52 published studies involving 291,413 patients. Injury 2009; 40(7): 692−7.
14. *Carretta E, Bochicchio V, Rucci P, Fabbri G, Laus M, Fantini MP.* Hip fracture: effectiveness of early surgery to prevent 30-day mortality. Int Orthop 2011; 35(3): 419−24.
15. *Radoicic D, Popovic Z, Slavkovic Z.* Operative treatment of hip fractures in the elderly in the first 12 hours of admission versus delayed surgery: assessment of mortality and length of hospitalization. Minerva Ortop Traumatol 2013; 64(2): 155−62.
16. *Lefaivre KA, Macadam SA, Davidson DJ, Gandhi R, Chan H, Broekhuyse HM.* Length of stay, mortality, morbidity and delay to surgery in hip fractures. J Bone Joint Surg Br 2009; 91(7): 922−7.
17. *Simunovic N, Devereaux PJ, Sprague S, Guyatt GH, Schemitsch E, Debeer J,* et al. Effect of early surgery after hip fracture on mortality and complications: systematic review and meta-analysis. CMAJ 2010; 182(15): 1609−16.
18. *Uzoigwe CE, Burnand HG, Cheesman CL, Aghedo DO, Faizi M, Middleton RG.* Early and ultra-early surgery in hip fracture patients improves survival. Injury 2013; 44(6): 726−9.
19. *Sharma S, Draviraj KP, Bhamra MS.* Primary total hip replacement for acute displaced subcapital femoral fractures with a minimal three-year follow-up. Hip Int 2005; 15(1): 33−7.
20. *Narayan KK, George T.* Functional outcome of fracture neck of femur treated with total hip replacement versus bipolar arthroplasty in a South Asian population. Arch Orthop Trauma Surg 2006; 126(8): 545−8.
21. *Džupa V, Pazdírek P, Pacovský V, Bartoníček J, Skála-Rosenbaum J.* Primary total hip arthroplasty for displaced intracapsular fracture of the femoral neck: medium-term functional and radiographic outcomes. Cent Europ J Med 2008; 3(2): 207−13.
22. *Jameson SS, Kyle J, Baker PN, Mason J, Deehan DJ, McMurtry IA,* et al. Patient and implant survival following 4323 total hip replacements for acute femoral neck fracture: a retrospective cohort study using National Joint Registry data. J Bone Joint Surg Br 2012; 94(11): 1557−66.
23. *Chaudhry H, Mundi R, Einhorn TA, Russell TA, Parvizi J, Bhandari M.* Variability in the approach to total hip arthroplasty in patients with displaced femoral neck fractures. J Arthroplasty 2012; 27(4): 569−74.
24. *Bhandari M, Devereaux PJ, Swiontkowski MF, Tornetta P 3rd, Obremskey W, Koval KJ,* et al. Internal fixation compared with arthroplasty for displaced fractures of the femoral neck. A meta-analysis. J Bone Joint Surg Am 2003; 85-A(9): 1673−81.
25. *Sebestyén A, Boncz I, Sándor J, Nyárády J.* Effect of surgical delay on early mortality in patients with femoral neck fracture. Int Orthop 2008; 32(3): 375−9.
26. *Holt G, Smith R, Duncan K, Finlayson DF, Gregori A.* Early mortality after surgical fixation of hip fractures in the elderly: an analysis of data from the scottish hip fracture audit. J Bone Joint Surg Br 2008; 90(10): 1357−63.
27. *de Palma L, Torcianti M, Meco L, Catalani A, Marinelli M.* Operative delay and mortality in elderly patients with hip fracture: an observational study. Eur J Orthop Surg Traumatol 2014; 24(5): 783−8.
28. *Blomfeldt R, Törnkvist H, Eriksson K, Söderqvist A, Ponzer S, Tidermark J.* A randomised controlled trial comparing bipolar hemiarthroplasty with total hip replacement for displaced intracapsular fractures of the femoral neck in elderly patients. J Bone Joint Surg Br 2007; 89(2): 160−5.

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Differential gene expression in patients with anal fistula reveals high levels of prolactin receptor

Diferencijacija genske ekspresije kod bolesnika sa analnom fistulom ukazuje na visoki nivo prolaktinskih receptora

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Abstract

Background/Aim. There are limited data examining variations in the local expression of inflammatory mediators in anal fistulas where it is anticipated that an improved understanding of the inflammatory milieu might lead to the potential therapeutic option of instillation therapy in complicated cases. The aim of the present study was to examine prolactin receptors (PRLR) as inflammatory markers and to correlate their expression with both the complexity of anal fistulas and the likelihood of fistula recurrence. **Methods.** Microarray was used to screen the differentially expressed gene profile of anal fistula using anal mucosa samples with hemorrhoids with age- and sex-matched patients as controls and then a prospective analysis of 65 patients was conducted with anal fistulas. PRLR immunohistochemistry was performed to define expression in simple, complex and recurrent anal fistula cases. The quantitative image comparison was performed combining staining intensity with cellular distribution in order to create high and low score PRLR immunohistochemical groupings. **Results.** A differential expression profile of 190 genes was found. PRLR expression was 2.91 times lower in anal fistula compared with control. Sixty-five patients were assessed (35 simple, 30 complex cases). Simple fistulas showed significantly higher PRLR expression than complex cases with recurrent fistulae showing overall lower PRLR expression than *de novo* cases ($p = 0.001$). These findings were reflected in measurable integrated optical density for complex and recurrent cases (complex cases, $8.31 \pm 4.91 \times 10^4$ *vs* simple cases, $12.30 \pm 6.91 \times 10^4$; $p < 0.01$; recurrent cases, $7.21 \pm 3.51 \times 10^4$ *vs* primarily healing cases, $8.31 \pm 4.91 \times 10^4$; $p < 0.05$). In univariate regression analysis, low PRLR expression correlated with fistula complexity; a significant independent effect maintained in multivariate analysis odds ratio [(OR) low to high PRLR expression = 9.52; $p = 0.001$)]. **Conclusion.** PRLR expression inversely correlates with anal fistula complexity. Further work must define the specificity of this finding and its relationship to other conventional mediators of inflammation.

Key words:
rectal fistula; receptors, prolactin; gene expression; immunohistochemistry; biological markers.

Apstrakt

Uvod/Cilj. Ispitivanja varijacija u lokalnoj ekspresiji medijatora inflamacije kod analnih fistula su ograničena, a očekuje se da bolje razumevanje ove oblasti može da doprinese razvoju potencijalnih terapijskih opcija poput uvođenja instilacione terapije kod komplikovanih slučajeva. Cilj ove studije bio je da se ispitaju prolaktinski receptori (PRLR) kao pokazatelji inflamacije i utvrdi korelacija njihove ekspresije sa kompleksnošću analnih fistula i verovatnoćom pojave recidiva. **Metode**. Pomoću metode *microarray* najpre je izvršena diferencijacija gena u uzorcima analne mukoze bolesnika sa hemoroidima (kontrolna grupa), a potom prospektivno i kod bolesnika sa analnim fistulama (n = 65). Obe grupe bolesnika bile su komparabilne u pogledu starosti i pola. Imunohistohemijska analiza PRLR učinjena je sa ciljem definisanja njihove genske ekspresije kod nekomplikovanih, kompleksnih i recidivantnih analnih fistula. Kvantitativno poređenje bojenih preparata postignuto je kombinovanjem intenziteta bojenja sa ćelijskom distribucijom čime je dobijen nizak i visok skor PRLR. **Rezultati**. Diferencijalni profil ekspresije nađen je za 190 gena . Ekspresija PRLR bila je 2,91 puta manja kod analnih fistula u poređenju sa uzorcima kontrolne grupe. U grupi sa analnim fistulama, od 65 bolesnika bilo je 35 sa nekomplikovanim i 30 sa kompleksnim fistulama. Kod nekomplikovanih fistula ekspresija PRLR bila je značajno viša nego kod kompleksnih slučajeva sa recidivantnim fistulama, a ukupno niža u odnosu na *de novo* fistule ($p = 0,001$). Ovi nalazi reflektovali su se u izmerenim vrednostima integrisane optičke gustine za kompleksne i rekurentne fistule (kompleksne fistule, $8,31 \pm 4,91 \times$

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10^4 *vs* nekomplikovane fistule, 12,30 ± 6,91 × 10^4 ; $p < 0,05$); recidivantne fistule, 7,21 ± 3,51 × 10^4 *vs* primarno izlečene fistule, 8,31 ± 4,91 × 10^4; $p < 0,05$). Univarijatna regresiona analiza pokazala je da je niska ekspresija PRLR bila u korelaciji sa kompleksnošću fistule, a ovo je se pokazalo i kao značajan nezavisan faktor u multivarijantnoj [odds ratio (OR) za nisku prema visokoj ekspresiji PRLR = 9,52; $p = 0,001$]. **Zaključak**. Ekspresija PRLR stoji u inverznoj korelaciji sa kompleksnošću analnih fistula. Dalja istraživanja trebalo bi da definišu specifičnost ovog nalaza i njegovu povezanost sa drugim konvencionalnim medijatorima inflamacije.

Ključne reči:
rektum, fistula; receptori, prolaktinski; geni, ekspresija; imunohistohemija; biološki pokazatelji.

Introduction

The successful management of complex anal fistulas continues to represent a significant surgical challenge [1]. Low and simple fistulas can be safely treated by fistulotomy [2] but complex cases may be managed with a wide variety of alternatives, each balancing long-term cure with reported postoperative continence disturbance [3–5]. Whereas it is believed that most fistulas develop from a cryptoglandular source, it is unclear why some cases become complicated or recurrent [6, 7] with most of these more specialized cases generally being separated in reported analyses from those with an underlying cause such as perianal Crohn's disease, ulcerative colitis, HIV-related sepsis or anorectal malignancy. It is speculated that the development of recalcitrant, recurrent cryptoglandular anal fistulas may be consequent upon a disturbed local immune microenviroment. Concerning this point, there are limited data examining variations in the local expression of inflammatory mediators in anal fistulas where it is anticipated that an improved understanding of the inflammatory milieu might lead to the potential therapeutic option of instillation therapy in complicated cases [8].

Prolactin (PRL) is one of a group of important stress hormones which has been shown to play an important role in local immune regulation where it has a specific immunostimulatory effect [9, 10]. The complex mechanisms by which PRL stimulates the proliferation of immune cells include an enhancement of mitogenic responses, the preservation of B and T lymphocyte function through antagonism of glucocorticoid-induced apoptosis [11, 12], the activation of macrophage phagocytosis and the induction of humoral IgM and IgG responsiveness and cell-mediated immunity [13]. Local regulation of PRL is mediated by specific PRL receptors (PRLR) which are expressed in many cells including those of the mammary gland, prostate, skin, decidua, brain and rectal mucosa [14]. In this regard, PRL and PRLR expression is likely to be upregulated in anal fistula, however, it is unknown if this is the case or whether ligand and receptor expression correlates with fistula chronicity. In our preliminary work, we found 190 genes of significant difference between the anal mucosa derived from patients with anal fistulas when compared with age- and sex-matched non-fistula controls. Among them, we noted differential PRLR expression (FC = 2.91, $p < 0.01$), suggesting a role for this molecular marker in the prediction of fistula complexity.

The aim of this study was to examine immunohistochemical PRLR expression within nearby anal canal mucosa of anal fistula and to correlate this tissue expression with clinical fistula type.

Methods

Patients

The study protocol was approved by the local hospital ethics committee and all patients signed an informed consent for study participation. Patients in this prospective analysis were obtained through the Department of Colorectal Surgery at the Third People's Hospital of Hangzhou China between June 2010 and September 2013. For the purposes of classification for this particular study, simple anal fistulas were defined as those with a single external and internal opening and a single straightforward fistula track. Complex fistulas were defined by two or more external or internal openings, two or more fistula tracks and those accompanied by secondary branching tracks [15]. Patient data were obtained from electronic medical records with a postoperative review at 6 weeks and subsequently at 3 monthly intervals until healing became evident or until the operation was deemed to be unsuccessful. Success was defined as closure of both the internal and external openings without persistent discharge. Time to failure was defined as the time from the fistula operation until clinical examination which confirmed fistula persistence or recurrence requiring further surgical intervention. A recurrent fistula was defined as one which had undergone at least one previous definitive operation. All patients underwent 3D endoanal sonography and appropriate gadolinium-enhanced magnetic resonance imaging. Patients with non-cryptogenic anal fistula (perianal Crohn's disease, ulcerative colitis and gastrointestinal tuberculosis) were excluded from analysis. All operations were performed in the prone jack knife position under spinal anaesthesia. Bowel preparation was used in all cases one day prior to scheduled surgery with all procedures performed by a consultant colorectal surgeon.

Gene expression microarray

Total RNA was isolated and purified from anal mucosa samples of three sex- and age-matched anal fistulas and hemorrhoids patients respectively, using QiagenRNeasy Mini Kit, QIAshredder kit and RNase-Free DNase Set kit (Qiagen, Valencia, CA) following manufacturer's recommendations. A total of 100 ng of each RNA was used to perform reverse transcription and one-color labeling steps. Amplified and labeled samples were purified using the RNeasy Mini Kit from Qiagen. Gene expression profiling was done with human whole-genome Agilent 28004, 8 × 60 K microarray chips following standard operating procedures from Agilent Tec-

hnologies. The labeled RNA was hybridized at 65°C for 17 hours at 10 rpm. Raw data files from Feature Extraction were imported into R with LIMMA, an R package from the Bioconductor project, and processed as follows: gMedianSignal data were imported, control probes were systematically removed, and flagged probes were set to Non Available. Interarray normalization was performed by quantile normalization. A single value was obtained for each transcript, taking the mean of each replicated probes summarized data. Missing values were inferred using k-Nearest Neighbors (KNN) algorithm from the package "impute" from R bioconductor. Normalized data were then analyzed with GeneSpring GX. To assess differentially expressed genes between two groups, we started by fitting a linear model to the data. Then we used an empirical Bayes method to moderate the standard errors of the estimated log-fold changes. The top-ranked genes were selected with the following criteria: an absolute fold change > 2 and an adjusted p value < 0.05.

Immunohistochemistry

At the time of surgery, biopsies were obtained for PRLR expression immunohistochemistry from the rectal mucosa above the dentate line and at least 5 mm away from the internal opening. All samples were analyzed by a single pathologist (Dr Lin) experienced in the assessment of anorectal specimens and blinded to the clinical and operative findings. Formalin-fixed paraffin-embedded tissue sections were deparaffinized and rehydrated, following which they were retrieved for heat-induced epitope retrieval. Endogenous peroxidase was inhibited with 3% hydrogen peroxide (H_2O_2) and non-specific antigen was blocked with 5% bovine serum albumin (BSA; Amresco, Solon, OH, USA). Slides were then incubated overnight with the primary antibody (Rabbit Anti-PRLR, Boster, China) at 4°C and then rinsed 3 times in phosphate-buffered saline (PBS) for 5 minutes at room temperature. Following this they were incubated with a biotinylated secondary antibody (diluted 1:100) and then incubated with a streptavidin–biotin peroxidase complex (diluted 1:100). Immunohistochemical detection was performed with 3,3′-diaminobenzidinetetrahydrochloride (DAB) in accordance with the manufacturer's instructions. Tissue sections were examined with a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with a camera. Images were captured using the NIS-Element S.F. 2.30 software at × 40, × 200, and × 400 magnification. Two techniques were used to analyze the immunohistochemical section. In the first method, 5 non-overlapping images were captured from each slide at × 400 magnification. Quantitative image analysis was performed using the Image-Pro Plus 6.0 software (Media Cybernetics, USA) and data were expressed as an integrated optical density (IOD) equal to the area × the average density of PRLR immunoreactivity identified, represented as the mean expression ± SD. In the second method, immunoreactivity was evaluated based upon the percentage of positively-stained cells combined with an estimate of staining intensity. For recording purposes, the percentage of positive cells was scored as 0 (< 10 %), 1 (10–40 %), 2 (40–70 %), or 3 (≥ 70 %) with the staining intensity being scored as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). The sum of the intensity and distribution score was used as the final staining score. Pathological sections with a final score of ≥ 3 were considered to have high PRLR expression and those < 3 as having low PRLR expression.

Statistics

Statistical analyses were performed with the SPSS v11.5 software (SPSS Inc.,Chicago, USA). Comparisons between groups were performed with the χ^2 test and the Fisher's exact method where appropriate. Assessment of immunoreactivity and comparisons of fistula complexity (as defined) was performed by a multiple logistic regression with values for two-sided significance < 0.05 considered as significant.

Results

We compared 36,788 genes between patients with anal fistulas and the control group, finding a differential gene expression profile of 190 genes (Figures 1 and 2). Among them we noted differential PRLR expression (FC = 2.91, p <

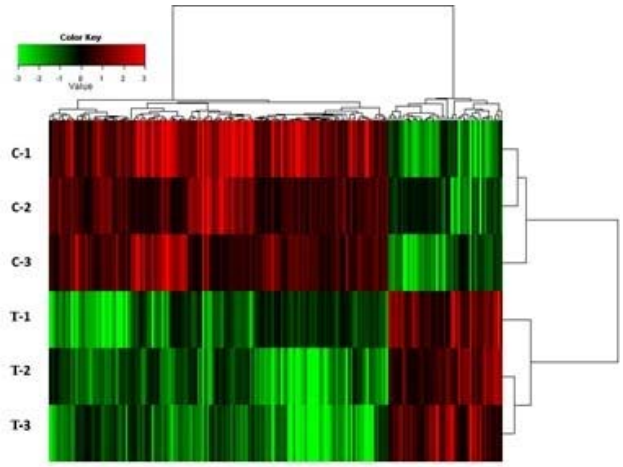

Fig. 1 – Cluster result of differentially expressed genes between anal fistula and control patients with Agilent human mRNA microarray. T1-3 – anal mucosa tissue of anal fistula patients; C1–3-control patients.

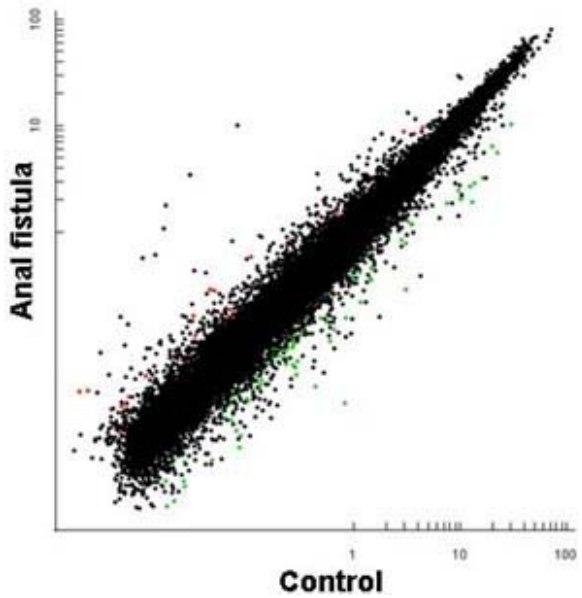

Fig. 2 – Scatter plot of differentially expressed genes between anal fistula and control patients with Agilent human mRNA microarray, (red dot – more than two folds up-regulation in anal fistula; green dot – more than two folds down-regulation in anal fistula; black dot – no significant difference).

0.01). Further analysis revealed the top three biological processes whose genes involved were keratinization, immune response and cell signaling.

Table 1 shows the clinicopathologic demographics for 65 patients assessed in the analysis including 35 simple (33 primary) and 30 complex (27 primary) cases (as defined). No differences were noted in the mean age of the patient groups, the mean body mass index (BMI) or in the time between diagnosis and surgery. Figure 3 shows examples of high and low PRLR expression in 2 rectal mucosal samples. Table 2 shows the separation into high and low PRLR expression groups where simple fistulas had significantly higher expression than complex cases ($p = 0.001$) and where low PRLR expression was more common in recurrent fistulas ($p = 0.001$). These findings were also reflected in the mean IOD measures where the recorded IOD for complex anal fistulas was significantly lower than that of simple cases ($8.31 \pm 4.91 \times 10^4$ *vs* $12.30 \pm 6.91 \times 10^4$, respectively; $p < 0.01$). The mean IOD measures of non-healing cases was lower than that in primarily healing fistulas ($7.21 \pm 3.51 \times 10^4$ *vs*. $8.31 \pm 4.91 \times 10^4$, respectively; $p < 0.05$). Univariate analysis showed that low PRLR expression was associated with fistula complexity but not with age, gender or the time between fistula diagnosis and surgery. The complexity of the fistula (as defined) was maintained in a multivariate analysis as an independent predictor for low fistula PRLR expression (odds ratio low to high PRLR score = 9.52; $p = 0.001$) (Table 3).

Table 4 presents list of highest rank of over-expressed and under-expressed genes in anal mucosa in patients with anal fistula.

Table 1

Demographic data for 65 patient with anal fistulas

Patient characteristics	Anal fistula		*p*
	simple	complex	
Number (n)	35	30	
Age (years), $\bar{x} \pm$ SD (range)	34.95 ± 12.18 (18–45)	32.45 ± 14.58 (21–54)	0.560
Gender (n)			
males	30	27	0.325
females	5	3	
Type of fistula (n)			
primary fistula	33	27	0.302
recurrent fistula	2	3	
BMI (kg/m^2), $\bar{x} \pm$ SD	24.32 ± 4.22	23.18 ± 5.38	0.452
Time between diagnosis and surgery (weeks), median (interquartile range)	14 (4–38)	20 (6–50)	0.152

$\bar{x}$ – mean; SD – standard deviation.

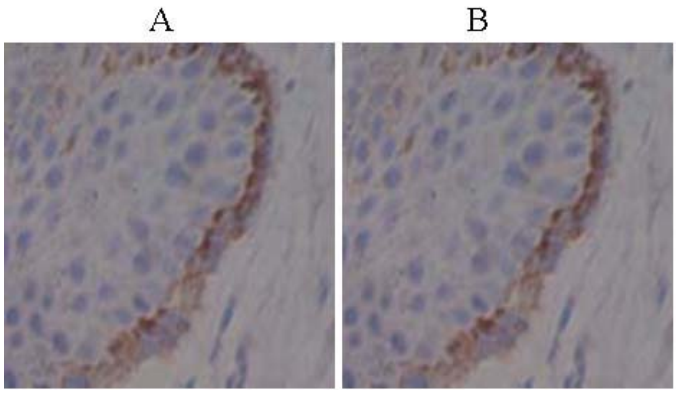

Fig. 3 – Representative microscopic appearance of the rectal mucosa in anal fistula biopsy specimens stained for prolactin receptor (PRLR): A) shows high PRLR expression, and B) demonstrates low PRLR expression (magnification ×200)

Table 2

Comparison of clinicopathological and fistula features with high or low prolactin receptors (PRLR) expression

Clinicopathological features		Score of PRLR expression		*p*
		high[a]	low[b]	
Number of patients (n)	65			
Age (years)				
< 40	41	17	24	0.913
≥40	24	13	11	
Gender (n)				
males	57	28	29	0.826
females	8	2	6	
Fistula classification (n)				
simple	35	30	5	0.001
complex	30	6	24	
Fistula recurrence (n)				
yes	5	1	4	0.001
no	60	35	15	

See Methods for definition of the intensity of PRLR immunoreactivity; [a] Score ≥ 3, [b] Score < 3.

Table 3

Univariate and multivariate analysis assessing the association between clinical features and fistula complexity

Clinicopathological features	Univariate analysis			Multivariate analysis	*p*
	n (%)	n (%)	*p*	OR	
Age, (years)					
< 40 *vs* ≥ 40	21 (32.3)	9 (13.8)	0.564		ns
Gender					
males *vs* females	27 (41.5)	3 (4.6)	0.236		ns
Time between diagnosis and surgery (weeks)					
< 24 *vs* ≥ 24	13 (20.0)	17 (26.1)	0.385		ns
PRLR expression groups					
low *vs* high	24 (36.9)	4 (15.4)	0.0001	9.520	0.001

ns – not significant; Low prolactin receptor (PRLR) expression – < 3; High PRLR expression – ≥ 3 (see Methods).

Table 4

List of highest ranked over-expressed and under-expressed genes in anal mucosa of anal fistula patients

Gene symbol	Fc (abs)	*p*
TREML1	6.41038	0.03559
UTY	9.945895	0.036397
CAMK1G	11.80925	0.000414
LOC643923	-9.164125	0.001235
LRCH1	-7.205685	0.039337
GPR116	5.369098	0.02471
DOK7	5.901879	0.04199
ELMOD1	5.434485	0.04398
IL37	10.87358	0.025695
LCE2D	7.009463	0.027215
PLB1	6.446507	0.014236
LCE5A	34.11338	0.00434

Fc (abs) – fold change, absolute.

Disscusion

As far as we are aware, this is the first report on the differentially expression profile of anal fistula and also the first recorded association between PRLR expression and anal fistula. This preliminary study shows that immunohistochemical PRLR expression is reduced in complex anal fistula cases when compared with simple fistulas. Low PRLR expression is also shown in non-healing cases confirming the qualitative findings with quantitative IOD measurements of the receptor. On multivariate analysis, fistula complexity was an independent predictor for low PRLR expression.

Currently there are limited available data concerning disturbances in the local peri-fistular inflammatory milieu of cryptogenic cases where it is anticipated that an improved understanding of the role of inflammatory mediators may explain why some fistulas become chronic, complex or fail to heal. PRLR which was originally identified as a lactotrophic hormone secreted by the pituitary has been implicated as an important immunomodulator under conditions of stress [16]. In this setting PRLR opposes the effects of glucocorticoids and other inflammatory mediators attenuating the acute phase response [17], with a regulatory effect on the generation of other inflammatory mediators [18]. During the acute phase response, PRL activity is typically suppressed [19] although the effects on the adaptive immune response during chronic inflammation on both PRL and its receptor have only been poorly characterized. The autocrine and paracrine interaction between the lactotrophes and other pituitary hormones, cytokines, inflammatory mediators and growth factors or with the other pituitary endocrine cell types (somatotropes, corticotropes, thyrotropes and gonadotropes) in simple or persistent surgical inflammatory conditions, remains to be elucidated [20]. Our study has shown that receptor expression is much higher in simple, healing anal fistula types probably consequent upon cytokine-mediated inhibition with coincident inhibition of its ligand. We believe a personalized surgery is very important when we try to address a fistula. Especially when it is a complex fistula, we have to weigh between the recurrence rate and potential chance of anal incontinence. Our study suggests that the molecular milieu of anal fistula might help us to evaluate the complexity and recurrence, which is useful for surgeons to make a reasonable decision about what operation to do for a specific anal fistula. Our study can also serve as a basis for a future attempt of pharmacological treatment of anal fistula by *ie* prolactin inhibitors or prolactin analogs.

This preliminary study has a number of significant limitations, however, the early findings are of sufficient interest as to demand further study. The small number of patients and the somewhat unexpected finding of PRL and PRLR association with anal fistula should be currently viewed with caution until larger number of patients is accumulated. The fistula classification system used in our study is unorthodox and more prolonged analysis along with magnetic resonance (MR) imaging may better define the association between PRLR expression and fistula outcome. It is accepted at this early stage that the findings may reflect the intensity of inflammation rather than show a specificity for high-risk anal fistula. It is currently unknown how the chronicity of inflammation in any part of the body influences local PRL and PRLR expression or the effect of glucocorticoids and catecholamines on PRL/PRLR dynamics within this orchestrated host response [21]. Planned future work will use an animal anal fistula model [22] so as to examine both the total PRLR mRNA expression and that of any different PRLR isoform transcripts which have previously been shown to differentially affect PRL signaling [23]. Comparison of anal fistula expression with other inflammatory tissues will be needed since stress responses to exogenously administered immunostimulants show that PRLR regulation is relatively tissue-specific [17]. Moreover, differences in PRLR expression will also reflect the differential activation of multiple promoters in the PRLR gene which will require molecular analysis [24].

Conclusion

Attenuation of the PRL/PRLR system appears to correlate with anal fistula complexity. It promotes our understanding of the occurrence of anal fistula, might help us to tailor the operation approach during preoperative evaluation of anal fistula and even provide rationale for a future attempt of pharmacological treatment of anal fistula. Further study is needed to examine and dissect the molecular genetics of the PRL/PRLR system in anal fistula.

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R E F E R E N C E S

1. *Steele SR, Kumar R, Feingold DL, Rafferty JL, Buie WD.* Standards Practice Task Force of the American Society of Colon and Rectal Surgeons. Practice parameters for the management of perianal abscess and fistula-in-ano. Dis Colon Rectum 2011; 54(12): 1465–74.
2. *Kronborg O.* To lay open or excise a fistula-in-ano: a randomized trial. Br J Surg 1985; 72(12): 970.
3. *Alasari S, Kim NK.* Overview of anal fistula and systematic review of ligation of the intersphincteric fistula tract (LIFT). Tech Coloproctol 2014; 18(1): 13–22.
4. *Lunniss PJ, Kamm MA, Phillips RK.* Factors affecting continence after surgery for anal fistula. Br J Surg 1994; 81(9): 1382–5.
5. *Garcia-Aguilar J, Belmonte C, Wong DW, Goldberg SM, Madoff RD.* Anal fistula surgery. Factors associated with recurrence and incontinence. Dis Colon Rectum 1996;39(7): 723–9.

6. *Sangwan YP, Rosen L, Riether RD, Stasik JJ, Sheets JA, Khubchandani IT*. Is simple fistula-in-ano simple? Dis Colon Rectum 1994; 37(9): 885–9.
7. *Pares D*. Pathogenesis and treatment of fistula in ano. Br J Surg 2011; 98(1): 2–3.
8. *Lewis R, Lunniss PJ, Hammond TM*. Novel biological strategies in the management of anal fistula. Colorectal Dis 2012; 14(12): 1445–5.
9. *Goffin V, Binart N, Touraine P, Kelly PA*. Prolactin: the new biology of an old hormone. Annu Rev Physiol 2002; 64: 47–67.
10. *Webster Marketon JI, Glaser R*. Stress hormones and immune function. Cell Immunol 2008; 252(1–2): 16–26.
11. *De Bellis A, Bizzarro A, Pivonello R, Lombardi G, Bellastella A*. Prolactin and autoimmunity. Pituitary 2005; 8(1): 25–30.
12. *Krishnan N, Thellin O, Buckley DJ, Horseman ND, Buckley AR*. Prolactin suppresses glucocorticoid-induced thymocyte apoptosis in vivo. Endocrinology 2003; 144(5): 2102–10.
13. *Xu D, Lin L, Lin X, Huang Z, Lei Z*. Immunoregulation of autocrine prolactin: suppressing the expression of costimulatory molecules and cytokines in T lymphocytes by prolactin receptor knockdown. Cell Immunol 2010; 263(1): 71–8.
14. *Bouchard B, Ormandy CJ, Di Santo JP, Kelly PA*. Immune system development and function in prolactin receptor-deficient mice. J Immunol 1999; 163(2): 576–82.
15. *Celi D, Desogus AI, Cucinotta A, Lucibello L, Giordano T, Metro D*, et al. Complex anal fistula with a recess above the levatorani muscles: report of a case complicated by haemorrhagic colitis. Chir Ital 2005; 57(4): 531–4. (Italian)
16. *Yu-Lee LY*. Prolactin modulation of immune and inflammatory responses. Recent Prog Horm Res 2002; 57: 435–55.
17. *Corbacho AM, Valacchi G, Kubala L, Olano-Martyin E, Schock BC, Kenny TP*, et al. Tissue-specific gene expression of prolactin receptor in the acute-phase response induced by lipopolysaccharides. Am J Physiol Endocrinol Metab 2004; 287(4): E750–7.
18. *Corbacho AM, Macotela Y, Nava G, Eiserich JP, Cross CE, Martinez de la Escalera G,* et al. Cytokine induction of prolactin receptors mediates prolactin inhibition of nitric oxide synthesis in pulmonary fibroblasts. FEBS Lett 2003; 544(1–3): 171–5.
19. *McCann SM, Kimura M, Karanth S, Yu WH, Mastronardi CA, Rettori V*. The mechanism of action of cytokines to control the release of hypothalamic and pituitary hormones in infection. Ann N Y Acad Sci 2000; 917: 4–18.
20. *Bilezikjian LM, Vale WW*. The local control of the pituitary by Activin signaling and modulation. Open Neuroendocrinol J 2011; 4: 90–101.
21. *Berczi I, Bertok L, Chow DA*. Natural immunity and neuroimmune host defense. Ann N Y Acad Sci 2000; 917: 248–57.
22. *Buchanan GN, Sibbons P, Osborn M, Bartram CI, Ansari T, Halligan S,* et al. Experimental model of fistula-in-ano. Dis Colon Rectum 2005; 48(2): 353–8.
23. *Das R, Vonderhaar BK*. Transduction of prolactin's (PRL) growth signal through both long and short forms of the PRL receptor. Mol Endocrinol 1995; 9(2): 1750–9.
24. *Hu ZZ, Zhuang L, Meng J, Leondires M, Dufau ML*. The human prolactin receptor gene structure and alternative promoter utilization: the generic promoter hPIII and a novel human promoter hP(N). J Clin Endocrinol Metab 1999; 84(3): 1153–6.

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Benign paroxysmal torticollis in infancy – diagnostic error possibility

Benigni paroksizmalni tortikolis u detinjstvu – mogućnost dijagnostičke greške

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Abstract

Background/Aim. Benign paroxysmal torticollis (BPT) is a rare paroxysmal dyskinesia characterized by attacks of head tilt alone or tilt accompanied by vomiting and ataxia, which may last hours to days. It is claimed that BPT disappears completely in childhood, but that it can evolve into other conditions, such as benign paroxysmal vertigo, cyclical vomiting syndrome, abdominal migraine, hemiplegic migraine, motion sickness and/or migraine with aura. The aim of this manuscript was to renew focus on benign paroxysmal torticollis because the disorder is almost always underrecognized by pediatric practitioners, who often order extensive and unrewarding testing and physiotherapy treatment. **Methods.** Twelve BPT cases observed during a 5-year period (2009–2014) at the Clinical Centre Niš, Niš, Serbia were reviewed. Data were collected on the features of torticollis, the age of onset, the duration of episodes, associated symptoms, the frequency of episodes, the persistence of symptoms over time, the age when the disorder finally disappeared, sequelae appearing after the 5th birthday, and family history of BPT, migraine or kinetosis. All the children were followed for periods ranging from 48 to 72 months. **Results.** The series included 6 females and 6 males. The age at onset of BPT was less than 8 months in 84% of the cases. Episodes of torticollis occurred suddenly on waking in all the cases without any trigger factors. The duration of torticollis ranged from a few hours to a few weeks. In 58% of cases, the condition persisted for more than one week. The frequency of the episodes ranged from once every 3 days to once every 25 days. The episodes were more frequent and lasted longer in the early months and tended to cease as the child became older. The age when episodes ended ranged from 11 months to 62 months. In 11 (91.66%) cases, the disorder disappeared before the patient's 5th birthday. No patient had a family history of BPT. In 6 cases, family members had kinetosis. In 5 cases, family members were positive for both migraine and kinetosis. All the children had normal motor development and normal speech and language development. After the disappearance of BPT, two children developed other forms of periodic syndromes: one boy had migraine with aura, and one girl experienced cyclic vomiting. **Conclusion.** BPT is probably an age-sensitive and migraine-related disorder that is benign in nature. The disorder is often misinterpreted, and children may pointlessly undergo numerous tests. Therefore, it is very important to recognize and observe this condition in order to avoid extensive, unnecessary and unpleasant procedures on the child.

Key words:
torticollis; diagnosis; diagnostic errors; child.

Apstrakt

Uvod/Cilj. Benigni parosizmalni tortikolis (BPT) je retka paroksizmalna diskinezija koja se manifestuje iznenadnom pojavom prinudnog položaja glave, izolovano ili udruženo sa povraćanjem i ataksijom. Smatra se da BPT kompletno iščezava u detinjstvu ili evoluira u druga stanja kao što su: benigni paroksizmalni vertigo, sindrom cikličnog povraćanja, abdominalna migrena, hemiplegična migrena, poremećaj pokretljivosti i/ili migrena sa aurom. Cilj ovog rada bio je da se fokus usmeri na BPT, jer ovaj entitet često ostaje neprepoznat u pedijatrijskoj praksi, pa se preduzimaju opsežne i nepotrebne dijagnostičke i terapijske procedure. **Metode.** Tokom petogodišnjeg perioda (2009–2014) u Kliničkom centru Niš, (Niš, Srbija), praćeno je dvanaestoro dece sa BPT. Prikupljani su podaci o obeležjima tortikolisa, starosti dece prilikom inicijalne pojave poremećaja, dužini epizoda i pridruženim simptomima, o učestalosti epizoda, starosti u kojoj je poremećaj iščezao, posledicama prisutnim nakon petog rođendana i podacima u vezi sa porodičnom anamnezom BPT, migrene i kinetoze. Sva deca su praćena tokom perioda od 48–72 meseca**. Rezultati.** Praćenjem je obuhvaćeno 6 dečaka i 6 devojčica. Prva pojava simptoma je kod 84% ispitanika bila pre 8. meseca života. Kod svih ispitanika epizode tortikolisa javljale su se iznenada i spontano prilikom buđenja. Tortikolis se ispoljavao u trajanju od

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nekoliko časova do nekoliko nedelja. Kod 58% ispitanika tortikolis se održavao više od jedne nedelje. Frekventnost epizoda kretala se od jednog pojavljivanja u tri dana do jednog pojavljivanja u 25 dana. Epizode su bile češće i duže su trajale u prvim mesecima života i imale su tendenciju prestanka sa odrastanjem deteta. Uzrast kada su se epizode sa tortikolisom završavale kretao se u rasponu od 11 do 62 meseca. Kod 11 (96%) ispitanika poremećaj je nestajao pre petog rođendana. Kod svih ispitanika nije postojala pozitivna porodična anamneza za BPT. Kod 6 ispitanika članovi porodice su imali kinetozu, dok je kod 6 ispitanika porodična anamneza bila pozitivna i za migrenu i kinetozu. Svi ispitanici su imali normalan razvoj motoričkih funkcija i sposobnosti govora. Nakon nestanka BPT, kod dvoje dece razvili su se drugi oblici periodičnih sindroma. Kod jednog dečaka ustanovljena je migrena bez aure, dok su se kod jedne devojčice ispoljila ciklična povraćanja**. Zaključak**. BPT je benigni poremećaj vezan za rano detinjstvo i često udružen sa migrenom. Obično se ne dijagnostikuje na vreme, pa se deca bespotrebno podvrgavaju brojnim ispitivanjima. Zbog toga je veoma važno blagovremeno prepoznati i pratiti ovo stanje čime bi se izbegle obimne, nepotrebne i neprijatne dijagnostičke i terapijske procedure.

Ključne reči:
tortikolis; dijagnoza; dijagnostičke greške; deca.

Introduction

Benign paroxysmal torticollis (BPT) is a rare paroxysmal dyskinesia characterized by attacks of head tilt alone or tilt accompanied by vomiting and ataxia, which may last hours to days [1]. It is an episodic functional disorder of unknown etiology that occurs in early infancy. It is characterized by recurrent episodes of abnormal rotation and inclination of the head to one side, which are sometimes accompanied by an asymmetric posture of the trunk with bending toward the same side (tortipelvis) [2]. Attacks can cause distress to the child and parents, but unfortunately, symptomatic treatment has not been helpful. The differential diagnosis may be difficult in case when the first attack occurs in a previously healthy infant. It includes seizures, vertigo, gastroesophageal reflux, idiopathic torsion dystonia, dystonic reactions to drugs, stroke, familiar hemiplegic migraine, alternating hemiplegia of childhood, ocular palsy, posterior fossa tumors, cervical spine abnormalities and Sandifer syndrome [3–5].

A study has been recently conducted with the aim of determining the general awereness of pediatricians of BPT. The study has shown that only 2 out of 82 pediatricians (2.4%) are aware of this condition [6].

The diagnosis of BPT in infancy is based on clinical grounds: a proper history and physical examination in paticular. Other examinations, such as neuroimaging, long-term video EEG recording or genetic studies, are necessary only if other diagnoses are suspected and other more serious conditions should be excluded.When the tests prompted by the first episode reveal negative findings, the parents relax and do not return to the pediatrician. The recurrent nature of the episodes is what leads to the diagnosis of BPT [2, 7].

Currently known facts about benign paroxysmal torticollis in infancy

There are relatively few reports on this disorder in the literature, with approximately 100 cases being reported since 1969. BPT was first described in 1969 by Snyder [8], who reported 12 cases with onset in infancy.

BPT is a self-limited condition that occurs in the first few months of life, usually between the age of 2 weeks and 5 months. The disorder is characterized by recurrent episodes of torticollis, which is often accompanied by vomiting, pallor, irritability, ataxia and drowsiness. Attacks can occur on either side and can last for hours or days, but most attacks last for less than a week. The episodes recur every few days to every few months. Improvement is seen by the age of 2 years and the episodes end by the age of 5. There is often a family history of migraine and motion sickness. BPT is a self-limited condition that usually does not respond to any kind of treatment. Sometimes, coordination problems, particularly gross motor difficulties, may accompany BPT, suggesting that the disorder is probably brain-based and maturational in nature [9].

It is crucial to recognise the condition and to reassure parents of its benign course and not to be misdiagnosed for other disorders.

BPT is not considered to be a frequent cause of torticollis. In 700 cases of primary torticollis (followed from 1970 to 1988), only 7 cases (1%) were due to BPT [10]. The etiopathogenesis of BPT is unknown. Some authors suggest an underlying vestibular disorder such as labyrinthitis [8]. Others claim the involvement of the central vestibular region or vestibulocerebellar connections [11]. It is also believed that the immaturity of the brain or a deficiency of certain neurotransmitters during a limited period of life is also involved.

The hypothesis of channelopathy has been raised. This entity has been recently linked to a mutation in the CACNA1A gene [12, 13]. Giffin et al. [12] suggested in 2002 that BPT was a migraine equivalent, related to familial hemiplegic migraine and connected to a CACNA1A mutation on chromosome 19. Recent studies have shown that heterogeneous paroxysmal disorders which include BPT are associated with PRRT2 gene mutation [14, 15].

Recognising the possible presence of a genetic defect in at least some cases of BPT, certain authors have suggested that BPT is a developmentally sensitive disorder associated with immaturity of the central nervous system.

Kimura and Nezu [16] reported on findings related to surface electromyography (s-EMG) in one case with BPT and suggested that BPT should be categorized as an idiopathic paroxysmal dystonia in infancy. The common finding of the family history of migraine and/or kinetosis supports the hypothesis that BPT is caused in such patients not only by an immaturity of the neuronal system, but also by a hereditary

predisposition for persistent functional vestibular dysfunction [17, 18]. Other authors reported a positive family history of migraine and kinetosis in 25–55% of cases [2, 18, 19].

It is claimed that BPT disappears completely in childhood, but that it can evolve into other conditions, such as benign paroxysmal vertigo, cyclical vomiting syndrome, abdominal migraine, hemiplegic migraine, motion sickness and/or migraine with aura [20, 21].

The aim of this manuscript was to renew focus on benign paroxysmal torticollis because the disorder is almost always under-recognized by pediatric practitioners, who often order extensive and unrewarding testing and physiotherapy treatment.

Methods

Twelve BPT cases observed during a 5-year period (2009–2014) at the Clinical Centre Niš, Niš, Serbia were reviewed. All the subjects were sent to pediatric rehabilitation department by pediatricians for treatment with physiotherapy. Data were collected on the features of torticollis, the age of onset, the duration of episodes, associated symptoms, the frequency of episodes, the persistence of symptoms over time, the age when the disorder finally disappeared, sequelae appearing after the 5th birthday, and family history of BPT, migraine or kinetosis. All the children were followed for periods ranging from 48 to 72 months.

Results and Discussion

The clinical features of included patients are presented in Table 1. The series included 6 females and 6 males.

The features of our cases are generally comparable with those of most cases described in the literature [22–24].

With respect to the age of onset, the frequency and duration of the episodes, associated symptoms and the age of disappearance, our results are similar to other reports [2, 10, 24]. The age at onset of BPT was less than 8 months in 84% of the cases. Episodes of torticollis occurred suddenly on waking in all the cases without any trigger factors. The duration of torticollis ranged from a few hours to a few weeks. In 58% of cases, the condition persisted for more than one week. The frequency of the episodes ranged from once every 3 days to once every 25 days. The head was always turned to the same side in 2 (16.67%) cases, but in 9 (75%) cases torticollis alternated sides with successive attacks.

Accompanying symptoms during attacks of torticollis included irritability (4 cases), vomiting (3 cases), pallor (4 cases) and drowsiness (5 cases). One child presented unstable gait during attacks at the age of 2.5 years. Tortipelvis was observed in 5 (41.67%) cases. The persistence of torticollis during sleep was observed in 2 cases. The episodes were more frequent and lasted longer in the early months and tended to cease as the child became older. The age when episodes ended ranged from 11 months to 62 months. In 11 (91.66%) cases, the disorder disappeared before the patient's 5th birthday. No patient had a family history of BPT. In 10 of the cases, first-degree or second-degree family members had family histories of migraine affecting at least one other family member (4 cases) or ≥ 2 family members (6 cases). In 6 cases, family members had kinetosis. In 5 cases, family members were positive for both migraine and kinetosis.

Table 1

The clinical features of the 12 patients with BPT in infancy

Clinical features	Patients, n (%)
Sex	
female	6/12 (50.00)
male	6/12 (50.00)
Age of onset (months)	
< 3	4/12 (33.33)
3–8	6/12 (50.00)
> 8	2/12 (16.66)
Duration of episodes (days)	
< 1	1/12 (8.33)
1–7	4/12 (33.33)
> 7	7/12 (58.33)
Always on the same side	2/12 (16.67)
Usually on the same side	1/12 (8.33)
Alternation between sides in successive episodes	9/12 (75.00)
Tortipelvis	5/12 (41.66)
Persistence during sleep	2/12 (16.66)
Accompanying symptoms	
irritability	4/12 (33.33)
vomiting	3/12 (25.00)
pallor	4/12 (33.33)
drowsiness/apathy	5/12 (41.67)
Age of disappearance (years)	
< 3	3/12 (25.00)
< 5	8/12 (66.67)
> 5	1/12 (8.33)
Family history of	
migraine	10/12 (83.33)
kinetosis	6/12 (50.00)

BPT – benign paroxysmal torticollis.

Instrumental tests including laboratory tests, brain ultrasound, orthopedic, ophthalmologic and otorhinolaryngologic examinations; ultrasound of the sternocleidomastoid muscle, and EEG were performed in all cases. Each test yielded normal results. The neurological findings were normal in all the cases, both during and after episodes, with no significant changes in muscle tone. A total of 5 (41.67%) children underwent magnetic resonance imaging of the brain. All the results were normal. All the children had normal motor development and normal speech and language development. After the disappearance of BPT, two children developed other forms of periodic syndromes: one boy had migraine with aura, and one girl experienced cyclic vomiting.

Conclusion

Benign paroxysmal torticollis is probably an age-sensitive and migraine-related disorder that is benign in nature. The disorder is often misinterpreted, and children may pointlessly undergo numerous tests. Therefore, it is very im-

portant to recognize and observe this condition in order to avoid extensive, unnecessary and unpleasant procedures on the child. The diagnosis of BPT in infancy is based on clinical grounds: a proper history and physical examination in paticular, and does not call for instrumental tests, which only cause pointless distress to the child and incur unnecessary expense and anxiety on the part of the parents.

The recurrent nature of the episodes in a previously healthy infant is what leads to the diagnosis of BPT.

In cases of torticollis which do not fit into the typical clinical picture of BPT, or if there are any additional symptoms, additional diagnostics is required in order to find the actual cause and avoid making a wrong diagnosis and losing precious time for the treatment of serious diseases.

REFERENCES

1. *Chaves-Carballo E.* Paroxysmal torticollis. Semin Pediatr Neurol 1996; 3(3): 255−6.
2. *Drigo P, Carli G, Laverda AM.* Benign paroxysmal torticollis of infancy. Brain Dev 2000; 22(3):169−72.
3. *Fernández-Alvarez E, Perez-Dueñas B.* Paroxysmal movement disorders and episodic ataxias. Handb Clin Neurol 2013; 112: 847−52.
4. *Caress JB, Nohria V, Fuchs H, Boustany RM.* Torticollis acquired in late infancy due to a cerebellar gangliocytoma. Int J Pediatr Otorhinolaryngol 1996; 36(1): 39−44.
5. *Rosman NP, Douglass LM, Sharif UM, Paolini J.* The neurology of benign paroxysmal torticollis of infancy: Report of 10 new cases and review of the literature. J Child Neurol 2009; 24(2): 154−60.
6. *Hadjipanayis A, Efstathiou E, Neubauer D.* Benign paroxysmal torticollis of infancy: An underdiagnosed condition. J Paediatr Child Health 2015; 51(7): 674−8.
7. *Dimitrijević L, Colović H, Zivković* V. Benign paroxysmal torticollis in infancy. Vojnosanit Pregl 2006; 63(1): 69−71. (Serbian)
8. *Snyder CH.* Paroxysmal torticollis in infancy. A possible form of labyrinthitis. Am J Dis Child 1969; 117(4): 458−60.
9. *Tomczak KK, Rosman NP.* Torticollis. J. Child Neurol 2013; 28(3): 365−78.
10. *Nikolić V, Banić M.* Paroxysmal torticollis in the developmental age. Med Pregl 1989; 42(3−4): 99−101. (Serbian)
11. *Cuvellier J, Lépine A.* Childhood periodic syndromes. Pediatr Neurol 2010; 42(1): 1−11.
12. *Giffin NJ, Benton S, Goadsby PJ.* Benign paroxysmal torticollis of infancy: Four new cases and linkage to CACNA1A mutation. Dev Med Child Neurol 2002; 44(7): 490−3.
13. *Vila-Pueyo M, Gené GG, Flotats-Bastardes M, Elorza X, Sintas C, Valverde MA,* et al. A loss-of-function CACNA1A mutation causing benign paroxysmal torticollis of infancy. Eur J Paediatr Neurol 2014; 18(3): 430−3.
14. *Dale RC, Gardiner A, Antony J, Houlden H.* Familial PRRT2 mutation with heterogeneous paroxysmal disorders including paroxysmal torticollis and hemiplegic migraine. Dev Med Child Neurol 2012; 54(10): 958−60.
15. *Méneret A, Gaudebout C, Riant F, Vidailhet M, Depienne C, Roze E.* PRRT2 mutations and paroxysmal disorders. Eur J Neurol 2013; 20(6): 872−8.
16. *Kimura S, Nezu A.* Electromyographic study in an infant with benign paroxysmal torticollis. Pediatr Neurol 1998; 19(3): 236−8.
17. *Uneri A, Turkdogan D.* Evaluation of vestibular functions in children with vertigo attacks. Arch Dis Child 2003; 88(6): 510−1.
18. *Al-Twaijri WA, Shevell MI.* Pediatric migraine equivalents: Occurrence and clinical features in practice. Pediatr Neurol 2002; 26(5): 365−8.
19. *Lanzi G, Balottin U, Fazzi E, Tagliasacchi M, Manfrin M, Mira E.* Benign paroxysmal vertigo of childhood: a long-term follow-up. Cephalalgia 1994; 14(6): 458−60.
20. *Winner P.* Migraine-related symptoms in childhood. Curr Pain Headache Rep 2013; 17(8): 339.
21. *Pacheva IH, Ivanov IS.* Migraine variants: Occurrence in pediatric neurology practice. Clin Neurol Neurosurg 2013; 115(9): 1775−83.
22. *Poddar U, Singh S, Kumar L.* Paroxysmal torticollis of infancy. Indian Pediatr 1999; 36(1): 86−8.
23. *Basheer SN.* Paroxysmal torticollis. J Pediatr Neurol 2010; 8: 69−71.
24. *Cohen HA, Nussinovitch M, Ashkenasi A, Straussberg R, Kauschansky A, Frydman M.* Benign paroxysmal torticollis in infancy. Pediatr Neurol 1993; 9: 488−90.

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Antiepileptic potential of ganaxolone

Antiepileptički potencijal ganaksolona

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Ključne reči:
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Introduction

With its estimated prevalence of 0.52% in Europe, 0.68% in the United States of America and up to 1.5% in developing countries, epilepsy makes a heavy burden on individuals, healthcare systems and societies in general all over the world [1, 2]. Despite long history of epilepsy treatment with medication, efficacy and effectiveness of available antiepileptic drugs as monotherapy were unequivocally proven in clinical trials only for partial-onset seizures in children and adults (including elderly), while generalized-onset tonic-clonic seizures in children and adults, juvenile myoclonic epilepsy and benign epilepsy with centrotemporal spikes are still waiting for optimal therapy [3–5]. It is estimated that 19–30% of epilepsy patients suffer from drug resistant epilepsy, which could not be controlled with available drugs, and they have to consider surgical treatment options [6–9]. Besides, antiepileptics are drugs with narrow therapeutic window, and control over epilepsy could easily be lost if the patients are switched from brand-name to generic, or from one to another generic antiepileptic. Although bioequivalence of generic drugs with their brand-name counterparts has to be confirmed prior to marketing authorization, generic antiepileptic drugs actually do not have the same bioavailability as brand-name drugs, and plasma concentration fluctuations could have much different pattern, leading to loss of seizures control [2–4]. Development of new antiepileptic drugs with mechanisms of action different from that of available anticonvulsants and with wide therapeutic window is one of the main ways to satisfy the unmet needs of patients with epilepsy. Ganaxolone, a positive allosteric modulator of gamma-aminobutyric acid-A (GABA-A) receptor, is one of the drugs with new mechanism of action which are currently in the process of clinical testing [2–4, 6, 7, 10].

New anticonvulsants

The drug resistant epilepsy has been recently defined by the International League Against Epilepsy as "a failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [11].The mechanisms of drug resistance in epilepsy are still incompletely understood, and none of the anticonvulsants with current marketing authorization has demonstrated superior efficacy in the treatment of drug resistant epilepsy [12]. Using new anticonvulsants as add-on therapy lead to freedom from seizures in only 6% of patients with drug resistant epilepsy [13]. This huge unmet need could be satisfied in the future only by synthesis and development of anticonvulsants with new mechanisms of action.

There are several anticonvulsants besides ganaxolone which are currently in the stage of clinical development: brivaracetam, seletracetam, talampanel, fluorofelbamate, carisbamate, and losigamone [14]. Being analogues of levetiracetam, brivaracetam and seletracetam bind with high-affinity for synaptic vesicle protein 2A (SV2A) and brivaracetam also inhibits voltage-gated sodium channels; talampanel is non-competitive allosteric blocker of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and weak inhibitor of kainite receptors for glutamate [15]. Fluorofelbamate has similar mechanism of action as felbamate, *ie* decreases responses of N-methyl-D-aspartate (NMDA) and kainate receptor to activation and blocks voltage-dependent sodium channels [15]. While the mechanism of carisbamate action remains unknown, losigamone is both sodium channel blocker, suppressor of NMDA-induced depolarization and enhancer of chloride uptake into neurons [15]. The mechanism of action of ganaxolone is different: it prevents seizures through positive allosteric modulation of synaptic

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and extrasynaptic GABA-A receptors, mimicking action of endogenous neurosteroids. Such subtle mechanism of action provided for beneficial efficacy/safety ratio of ganaxolone.

The compound

Ganaxolone is a synthetic molecule with the steroid backbone which resembles endogenous neurosteroids that are synthesized and acting in the brain. There are three groups of endogenous neurosteroids: pregnane neurosteroids (allopregnanolone and allotetrahydrodeoxycorticosterone), androstane neurosteroids (androstanediol and etiocholanolone) and sulfated neurosteroids (pregnenolone sulfate). Neurosteroids from the pregnane group show anti-seizure activity in various animal models due to their positiveallosteric modulation of GABA-A receptors and increase of chloride influx [16]. The development of ganaxolone was initiated by Kelvin Gee and Nancy Lan at CoSensys company [17], and then continued by Edward Monaghan and his associates [18]. Later development took place at Marinus Pharmaceuticals, Inc., after this company obtained development and commercialization rights from Purdue Pharmaceuticals, who acquired CoSensys in 1998 [18]. Ganaxolone is currently in phase 3 clinical trials, and the results are expected in 2016.

Chemistry

Reduction of progesterone at the 5- and 3-positions of the steroid A-ring leads to formation of endogenous neurosteroid allopregnanolone (through an intermediate metabolite 5α-dihydroprogesterone) which has modulating effect on GABA-A receptors, and lacks progestogenic effect [19]. However, allopregnanolone could be oxidized back to 5α-dihydroprogesterone which has progestogenic properties. Ganaxolone (3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one) is synthesized from allopregnanolone by methylation at position 3 of its A-ring (Figure 1). The methyl group added prevents conversion of ganaxolone back to a steroid with hormonal (progestogenic) properties, improving its safety profile.

Ganaxolone (molecular weight 332.52) is present at room temperature as white powder which is insoluble in water [20]. Due to its insolubility in water, significant efforts were made to prepare suitable oral formulations of ganaxolone with acceptable bioavailability. Successful formulation efforts resulted in a patent issued to Marinus Pharmaceuticals Inc. covering an oral suspension and capsule formulation. The new formulations achieved bioavailability of 300–400% compared to conventional ganaxolone formulations [21].

Pharmacodynamics

Mechanism of action

Allopregnanolone is a positive allosteric modulator of action of GABA on its A-type receptors, but various part of the brain differ in rate of allopregnanolone synthesis. Tissue concentrations of allopregnanolone are higher in mice olfactory bulb than in frontoparietal cortex or cerebellum. The study on patch-clamped neocortical pyramidal neurons of mice showed that blocking synthesis of allopregnanolone decreases chloride ion currents elicited by GABA-A receptor agonist muscimol; this effect was reversed with addition of allopregnanolone [22–24]. The mechanism of action of ganaxolone is similar to the mechanism of action of endogenous neurosteroid allopregnanolone: it binds for unique recognition site on the GABA-A receptor which is different from the binding sites of GABA, benzodiazepines and barbiturates. After binding, ganaxolone probably potentiates inhibitory action of GABA on neurons which carry the GABA-A receptor. *In vitro* studies on xenopus oocytes expressing the human GABA-A receptors showed that ganaxolone increased chlorine influx only after γ-aminobutyric acid exhibited its basal activity, while direct effect in the absence of GABA was of minor extent. Although action of ganaxolone was not dependent on subunit composition of the GABA-A receptor in this study (it was exhibited across all three GABA-A receptor subtypes tested: α1β2γ2, α2β2γ2, and α3β2γ2) [25], numerous other studies have shown that delta subunit enhances sensitivity to neurosteroids including ganaxolone. Neurosteroids bind for two sites on alpha

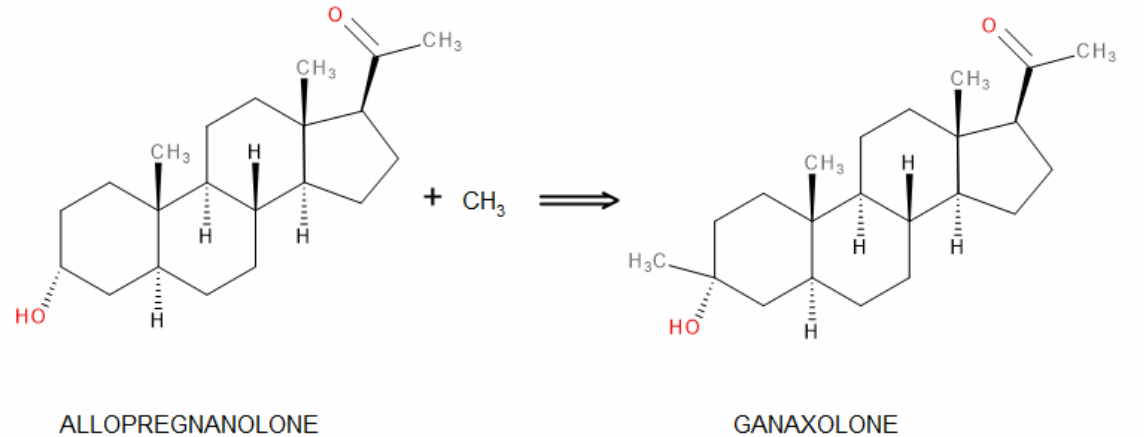

Fig. 1 – Chemical structures of ganaxolone and its precursor allopregnanolone.

subunit of GABA-A receptor: one is in located in transmembrane domain and is essential for potentiation of responses to GABA, and another is placed on contact surface between alpha and beta subunits, causing activation of the receptor. However, the GABA-A receptor could be activated only after both sites have been occupied by a neurosteroid [26].

Anti-epileptic effects

In a variety of animal models of epilepsy ganaxolone shows potent anti-seizure activity which is comparable to that of valproate: it prevents pentylenetetrazol (PTZ)-induced seizures in mice and rats, and bicuculline, tert-butylbicyclophosphorothionate (TBPS) or aminophylline-induced seizures in mice. In a rat cornea-kindled seizures model ganaxolone efficiently prevents seizures, and it significantly elevates seizure threshold in mice receiving pentylenetetrazol (PTZ) [25, 27]. Besides anticonvulsant activity against PTZ-induced clonic and tonic seizures in mice, ganaxolone shows anti-epileptogenic action against sensitization of the kindled mice to the convulsive and lethal effects of PTZ; its efficacy in this animal model was better than that of diazepam and valproate [28]. Both anticonvulsant and anti-epileptogenic effects of ganaxolone were recorded in cocaine-kindled seizures in male mice, too; this dual action gives important advantage to ganaxolone over conventional anticonvulsive drugs which mostly lack anti-epileptogenic action [29]. Ganaxolone was more potent than diazepam in exhibiting protection against cocaine-induced seizures in mice; when co-administered with diazepam, it acts synergistically to protect against both cocaine and pentylentetrazol-induced seizures in mice. Although high doses of ganaxolone produce motoric impairment similar to that induced by diazepam, the same was not observed at lower doses of ganaxolone which produce anticonvulsant action [27]. Beneficial ratio was also observed between doses of ganaxolone that prevent prolongation of cortical epileptic after discharges in rats caused by low-frequency stimulation of the sensorimotor cortical area through epidural electrodes, and doses that compromise motor activity, suggesting acceptable safety profile of the drug [30]. In a model of primarily generalized seizures in developing rats, where seizures were induced by inhalation of flurothyl, ganaxolone showed dose-dependent anticonvulsant effect [31].

Protective effect of ganaxolone against seizures was also shown in an animal model of infantile spasms. The rats were at first prenatally primed with betamethasone, and then on the day 15 after birth convulsions were initiated with NMDA. When given 30 min before the NMDA, ganaxolone delayed the onset of spasms and decreased the number of spasms or suppressed their occurrence [32].

In a mice model of complex partial seizures induced by low-frequency (6 Hz), long-duration (3 s) electrical stimulation ganaxolone showed strong protective effect, comparable to that of clonazepam. Potency of ganaxolone in this model was similar to its potency in models of PTZ-induced seizures (ED_{50} value = 6.3 mg/kg) [33]. The same protective effect with almost identical potency (ED_{50} value = 6.6 mg/kg) ganaxolone exerted in fully amygdala-kindled female mice (by means of the electrodes implanted into the right amygdala complex). The seizures were nearly completely prevented with the highest doses of ganaxolone, and its effect was comparable to protective effect of clonazepam. The potential advantage of ganaxolone over benzodiazepines lies in the absence of tolerance for protection against seizures, which is regularly observed in experiments with the latter drugs [34, 35].

Ganaxolone showed a specific protective anticonvulsive effect in a rat model of catamenial epilepsy (a kind of epilepsy with the exacerbation of seizures immediately before, during or after menstruation), which is believed to be caused by perimenstrual decrease in brain levels of progesterone metabolite allopregnanolone [36]. Almost 70% of women in reproductive age with epilepsy experience increase in seizure frequency around menstruation. Female rats were maintained at high levels of progesterone, then subsequently deprived of allopregnanolone by administration of finasteride. Ganaxolone gave protection against PTZ-induced seizures in much lower doses (*ie*, with higher potency) than in non-deprived pregnant or non-pregnant animals. Similar phenomenon was not observed with diazepam or valproate, which indicated that ganaxolone could be specific and potent drug for treatment of catamenial epilepsy in humans. Greater efficacy of neurosteroids including ganaxolone compared to benzodiazepines in the treatment of catamenial epilepsy in animal models could be explained by temporary increase in expression of delta subunit of GABA-A receptor caused by progesterone. GABA-A receptors which contain a delta subunit are located mostly perisynaptically/extrasynaptically, and GABA is less efficacious at such receptors. While benzodiazepines require gamma-2 subunit to act on GABA-A receptor, neurosteroids positively modulate GABA-A receptors with all kinds of subunits, especially those with delta-subunits which are more sensitive to them. These temporary changes in the composition of GABA-A receptors during and around menstruation give to neurosteroids a unique opportunity to enhance inhibitory effect of GABA and prevent exacerbation of seizures during and around menstruation [37].

On the other hand, ganaxolone was not only ineffective in animal models of absence seizures, but it showed seizure-potentiating activity. Pretreatment of rats with ganaxolone prolonged absence seizures caused by low-doses of PTZ or gamma-hydroxybutyric acid (GHB), and ganaxolone alone (> 20 mg/kg) caused occasional bilateral synchronous spike wave complexes in EEG [38]. When during *in vivo* experiments ganaxolone was focally micro-injected into WAG/Rij rats, genetically modified animals that suffer from absence-like epilepsy, with characteristic recordings of spike-wave complexes, it significantly increased frequency of spike-wave complexes when injected into thalamic but not somatosensory cortical nuclei [39] (Table 1).

Other central effects of ganaxolone

An anxiolytic-like effect of ganaxolone was observed on pentylentetrazol-treated mice: ganaxolone administered 15 minutes before pentylentetrazol prevented PTZ-induced

Table 1

Anti-epileptic effects of ganaxolone observed in pre-clinical studies

Experimental model	Observed effects
PTZ - induced seizures in mice	Elevates seizure threshold [27]
Rat cornea - kindled seizures model	Prevents seizures [25]
PTZ - induced seizures in kindled mice	Prevent sensitization of the kindled mice to PTZ [28]
Cocaine - kindled seizures in male mice	Anticonvulsant and anti-epileptogenic actions [29]
Primarily generalized seizures in developing rats induced by flurothyl	Dose-dependent anticonvulsant effect [31]
Rat model of infantile spasms	Prevents convulsions induced by NMDA [32]
Mice model of complex partial seizures	Prevents the seizures [33]
Fully amygdala - kindled female mice	Prevents complex partial seizures [34, 35]
Rat model of catamenial epilepsy	Prevent the seizures [36]
Absence seizures caused by low-doses of PTZ or gamma-hydroxybutyric acid	Ineffective [38]
WAG/Rij rats model of absence seizures	Increases frequency of spike-wave complexes [39]
Audiogenic seizures in Fmr1 knockout mice	Anti-convulsant effect [40]

PTZ – pentylenetetrazol; WAG/Rij – Wistar Albino Glaxo/Rij; NMDA – N-methyl-D-aspartate.

decrease in behaviors like sitting or lying without moving, lying with at least one of the back limbs clearly visible, the occurrence of small twitches of the body, the tail straightened backwards or pressed along a wall of the cage and sitting or lying with the nose turned to the corner of the cage. When compared with well-established anti-convulsants (phenobarbital, ethosuximide, clonazepam, diazepam and valproate), ganaxolone was more efficient in prevention of PTZ-induced behaviors than the majority of comparators except phenobarbital. The effect of ganaxolone is dose-dependent, and it occurs within the dose range that is not associated with motor toxicity like circling or uncoordinated walking [41]. Ganaxolone produced similar effects in mice treated by other pro-convulsive and anxiogenic drugs: it reversed locomotor depression caused by bicuculline, picrotoxin and yohimbine (it was the least potent against yohimbine) [42]. Interestingly, ganaxolone failed to decrease cocaine-induced hyperactivity in mice and motor stimulation caused by methamphetamine, dizocilpine, and phencyclidine, which suggests complex and regionally specific role of neurosteroids in control of locomotion [43]. Indeed, in a study on rat hippocampal CA1 pyramidal neurons and dentate granule cells using whole-cell patch-clamp recordings it was shown that the cells from the two hippocampal regions are differentially sensitive to neurosteroids in regard to enhancement of GABA-A receptor conductance due to both variations of the subunit composition and phosphorylation of the GABA-A receptor, and the differences in local steroid metabolism [44].

A possible antidepressant action of ganaxolone was hypothesized after *in vivo* experiments on dorsal raphe nucleus serotonergic neurons in female rats, where ganaxolone and endogenous neurosteroid allopregnanolone strongly increased spontaneous firing activity. When co-administered with a serotonin-uptake inhibitor citalopram, ganaxolone prevented the reduction of firing activity usually caused by citalopram after 3 or more days of treatment. This observation sets rationale for further testing of augmenting properties of ganaxolone in regard to the antidepressant effect produced by selective serotonin reuptake inhibitors [45]. Interestingly, fluoxetine and fluvoxamine have been shown to increase allopregnanolone levels at doses below those effective at serotonin transporters [46].

Ganaxolone, as well as endogenous neurosteroids, has certain effect on regulation of ethanol consumption in experimental rats. When administered systemically to rats trained to self-administer ethanol, ganaxolone at first shortens the latency until the animals start licking ethanol, and then decreases overall ethanol consumption [47–49]. The same effect was achieved after stereotaxic infusion of ganaxolone to nucleus accumbens shell [50], and it results from positive modulation of both synaptic and extra-synaptic GABA-A receptors. On the other hand, ganaxolone induces reinstatement of ethanol seeking behavior in mice that previously were trained to self-administer alcohol, and then extinguished [40]. These effects of ganaxolone should be taken into account if this drug is going to be used in patients with epilepsy and concomitant alcohol dependence.

A number of new areas where ganaxolone could offer therapeutic benefit were recently studied through animal models of fragile X syndrome, posttraumatic stress disorder, spinal analgesia, Niemann-Pick Type C disease and multiple sclerosis. In rodents (and humans) with the fragile X mental retardation gene (Fmr1) mutation the ensuing intellectual disability is accompanied with down-regulation of GABA transmission (decreased synthesis and increased catabolism of GABA, decreased number of GABA receptors). Ganaxolone effectively rescued audiogenic seizures in Fmr1 knockout mice through its positive modulation of GABA-A receptors [51]. In mice with mutation in the NPC1 gene and signs and symptoms which resemble Niemann-Pick Type C disease in humans activity of the neurosteroidogenic enzymes is decreased; administration of allopregnanolone or ganaxolone in such mice delay the onset and progression of neurological symptoms [52]. As the level of allprenanolone in cerebrospinal fluid is reduced in premenopausal women with post-traumatic stress disorder [53], beneficial effect of ganaxolone on this disorder was supposed by researchers who tried it on socially isolated (SI) mice, which have allop-

regnanolone deficiency and post-traumatic stress-like behaviors. Brain areas of the SI mice which control emotions (frontal cortex, hippocampus and basolateral amygdala) have decreased allopregnanolone levels, and GABA-A receptors on neurons in these areas have distinct subunit composition, with the decreased presence of gamma 2, alpha 1 and alpha 2 subunits. Such GABA-A reeptors are less sensitive to benzodiazepines, but retain sensitivity to neurosteroids, including ganaxolone. Unlike benzodiazepines, ganaxolone improved anxiety, aggression, and other posttraumatic stress disorder (PTSD) like behaviors in SI mice, without causing sedation or locomotor impairment [54, 55].

In models of pain, peripheral nerve injury causes changes in K(+)/Cl(-) cotransporter isoform 2 (KCC2) expression on spinal neurons from the dorsal horn; these changes lead to accumulation of chloride ion within the neurons, decreasing the flux of that ion through GABA-A channels, preventing hyperpolarization of neuronal membrane and creating allodynia (hypersensitivity). Intrathecal administration of ganaxolone within the framework of tail flick assay produces significant analgesic effect [56]. Finally, it has been shown in human material from multiple sclerosis patients as well as in mice with induced experimental autoimmune encephalomyelitis (EAE) that neuroinflammation increases expression of GABA transporter type 2, which then decreases concentration of extracellular GABA. On the other hand, GABA and ganaxolone decrease expression of receptors for inflammatory mediators on surface of activated macrophages, improving behavior of the animals and reducing demyelination and injury of nerve fibers [57] (Table 2).

metabolite 16α-hydroxyganaxolone. Only 20% of dose is eliminated through kidneys, and the rest is eliminated in feces [10]. The elimination half-life is approximately 10–30 h based on formulation and dose tested [1, 58].

It has linear kinetics of elimination, and after repeated dosing 500 mg three times a day (*tid*) steady-state was achieved after 48 h (significant accumulation of ganaxolone was not observed in clinical trials) [58]. Maximal ganaxolone concentrations in the steady state range from 32 ng/mL (the dosing regimen of 50 mg *bid*) to 376 ng/mL (the dosing regimen of 500 mg *bid*) with early formulations of oral suspension [59]. The newer formulation,, on oral capsule has maximum concentration (Cmax) of 239 ng/mL while maintaining area under the curve (AUC) [58], which may have a positive impact on tolerability. Total clearance of ganaxolone was not affected by creatinine, urea or aminotransferases serum levels according to population pharmacokinetic analysis from a clinical trial [60].

Ganaxolone neither induces nor inhibits activity of CYP3A4/5. However, strong inducers (*eg* carbamazepine) and inhibitors (*eg* ketoconazole) of CYP3A4/5 may increase and decrease clearance of ganaxolone, respectively [61].

Clinical efficacy

Phase I studies

Safety, tolerability, and pharmacokinetics of ganaxolone were reported from 7 phase I studies conducted on 87 healthy adult male and 9 healthy adult female volunteers [59]. The first study was open-label, single dose study on 15 male volunteers, testing the following oral doses: 50, 150, 300, 450, and 600 mg. In the second study on 16 volunteers higher single doses (900, 1,200 and 1,500 mg) of ganaxolone were tested using a double-blind, placebo-controlled design. The first-single-then-multiple doses design was used in two studies, one being double-blind, placebo-controlled (12 volunteers, 50, 200, and 500 mg/day), and another open-label study (6-volunteers, 300 mg *bid*). The excretion pathways and pharmacokinetics were studied on 6 male volunteers receiving single oral 300 mg dose of [^{14}C]-ganaxolone. The differences in ganaxolone pharmacokinetics

Table 2

Other central effects of ganaxolone observed in pre-clinical studies

Experimental model	Observed effects
Behaviors of pentylentetrazol-treated mice	Anxiolytic-like effect [41]
Locomotor depression of mice by bicuculline, picrotoxin and yohimbine	Reversal of the depression [42]
Cocaine-induced hyperactivity in mice	Ineffective [43]
Electrical activity of dorsal raphe nucleus serotonergic neurons in female rats	Increases spontaneous firing activity [45]
Rats trained to self-administer ethanol	Decreases overall ethanol consumption [46–49]
Mice model of Niemann-Pick Type C disease	Delays onset and progression of neurological symptoms [51]
Socially isolated (SI) mice model of post-traumatic stress disorder	Improves anxiety, aggression, and other PTSD-like behaviors [53, 54]
Tail flick assay in rats as model of nerve injury	Analgesic effect [55]
Autoimmune encephalomyelitis in mice	Decreases expression of receptors for inflammatory mediators and reduce demyelination [56]

PTSD – posttraumatic stress disorder.

Pharmacokinetics

After oral administration ganaxolone is rapidly and completely absorbed from the gastrointestinal tract: maximal plasma concentration after single oral dose is achieved after 1 to 4 hours [21]. Food increases bioavailability of ganaxolone which is formulated with submicron particulates in suspension or capsules, and area under the curve plasma concentration/time when ganaxolone is taken with food is 1.5 to 3 times greater than when it is taken on empty stomach [21]. Ganaxolone is 99% bound to plasma proteins and metabolized in the liver, by cytochromes CYP3A4/5, to an inactive

among sexes were tested in double-blind, single oral dose (300 or 900 mg), cross-over trial involving 8 males and 9 females. Finally, the seventh study assessed influence of food on absorption of ganaxolone, using different formulations (24 volunteers).

These studies show that ganaxolone in beta-cyclodextrin formulations has linear pharmacokinetics at the doses tested with rapid absorption and bi-exponential elimination, characterized by shorter initial, and a long terminal half-life (18–28 h and 37–70 h, respectively); reports with a newer formulation state a 7–10 h initial half-life [58]. The drug does not accumulate in the body after multiple doses. The safety of ganaxolone in these early studies was excellent. The only serious complaint of the volunteers was somnolence, but it was pronounced only after the highest doses (900, 1,200 and 1,500 mg), which could have been expected taking into account the mechanism of action of ganaxolone. No serious adverse events were recorded in the phase I studies.

Phase II studies

The first clinical trial on adult patients was conducted on 52 adults with a history of complex partial seizures with or without secondary generalization, who have been withdrawn from antiepileptic drugs during diagnostic evaluation for surgical treatment of seizures [61]. The study was double blind, randomized, placebo controlled, and lasted for 8 days. The patients started to take orally either ganaxolone (500 mg *tid* on the day 1 and 625 mg *tid* on the days 2 to 8) or placebo only after plasma concentrations of previously used anti-convulsive drugs dropped to the levels less than 25% of lower limit of the therapeutic range. The primary measure of antiepileptic activity was duration of treatment before withdrawal from the trial. Fifty percent of the patients treated with ganaxolone completed the study, in comparison with 25% of the patients on placebo. However, the study was underpowered to prove a significant difference ($p = 0.0795$) in the duration of treatment before withdrawal from the study due to one of the following: four seizures of any type except simple partial, three generalized tonic-clonic seizures in the patients who had such seizures before and one in the patients without such experience, and status epilepticus. Ganaxolone was well tolerated, with similar pattern and frequency of adverse events in the two groups. It was also observed that through plasma concentrations of ganaxolone did not correlate with anti-convulsive effect though most responders had trough levels above 20 ng/mL.

Ganaxolone was also tested in a small open-label study in children (7 months to 7 years old) with either refractory infantile spasms or continuing seizures after treatment for infantile spasms [62]. Ganaxolone was added to existing anti-convulsive therapy for 12 weeks, in oral doses that were gradually increased up to 36 mg/kg/d. The frequency of spasms was reduced for 50% or more in 33% of the patients, while another 33% of the patients had 25–50% less spasms. The patients did not experience any serious adverse event that could be attributed to ganaxolone.

The study with children 4 to 24 months of age suffering from infantile spasms and already treated unsuccessfully with 3 anti-epileptic drugs was designed as double-blind, placebo-controlled and randomized study. In total 56 patients participated in the study for two weeks, and then the study was extended for further 99 weeks in an open-label manner. The outcomes set before the study were not significantly different between the groups, but there were beneficial trends toward decrease in seizure clusters, better responder rates, global assessment of the patients and decrease of hypsarrhythmia [58].

Another small, open-label study with ganaxolone was conducted on 15 children 5–15 years of age with partial or generalized seizures (myoclonic seizures and epileptic spasms, too) that were not controlled with 2 anti-epileptic drugs [63]. Ganaxolone was given as add-on oral therapy, and in the first 16 days the doses were titrated from 1 mg/kg *bid* up to the maximal tolerated dose or to 12 mg/kg, *tid*; after the titration period, the patients were receiving the last titrated dose for the next 8 weeks. Although only 8 patients completed this study, an intention-to-treat analysis showed that after 8 weeks 27% of the patients had more than 50% reduction in the seizures frequency (responders), and 13% of the patients between 25 and 50%. Three of the responders continued to take ganaxolone, from 4 months to 3.5 years, maintaining the same level of response. The most frequent adverse events were somnolence (9 patients), convulsions (3 patients) and agitation (2 patients).

There is only one published clinical study (*ie*, case report) on usage of ganaxolone in catamenial epilepsy. Oral ganaxolone (300 mg/day, *bid*) was given to two women from the 21st day of the menstrual cycle to the third day of the menstruation, and it decreased the number of seizures [64].

A larger phase II clinical study, double-blind, placebo controlled and randomized, was conducted with 147 patients (100 women and 47 men,18 to 69 years old) suffering from partial onset seizures with or without secondary generalization and refractory to previously used anti-convulsants. The dose of oral ganaxolone was 500 mg/8 hours. During the 10-weeks study ganaxolone decreased the mean seizure frequency *per* week for 18%, while placebo increased it for 2%. In the ganaxolone group there were 26% of the patients with more than 50% reduction in seizure frequency, and in the placebo group only 13%. The study was extended in a way that the patients from both placebo and ganaxolone group continued to take ganaxolone, and even 38 patients remained in the extension phase for more than 52 weeks. Even 24% of all the patients had a decrease in seizure frequency for more than 50% in the extension study. The only adverse events which were more frequent in the ganaxolone group were somnolence (13% *vs* 2%), dizziness (16% *vs* 8%) and fatigue (16% *vs* 8%) [60, 65].

Phase III studies

There is only one phase III clinical trial with ganaxolone, which is currently ongoing, and its completion is planned for the year 2016. It is a multicenter, randomized, double-blind and placebo-controlled study investigating efficacy and safety of ganaxolone as add-on antiepileptic therapy for adult patients with partial-onset seizures that were not controlled with previous therapy. In Cohort 1, the study lasts for 9 weeks, and then the patients will enter open-label phase for 52 weeks. In the first 9 weeks, placebo or two doses of ganaxolone will be tested (1,200 mg/day and 1,800

mg/day), and in the open-label phase the dose of 1,800 mg/day open label ganaxolone will be used. A second cohort will receive 1,800 mg/day ganaxolone or placebo for 12 weeks and then enter the one-year open label phase. Primary outcome of the study is the percentage change in seizure frequency *per* 28 days relative to baseline in the second cohort, and the outcomes in the open-label phase are the change in seizure frequency, responder rate, percentage of seizure-free patients, change in percentage of seizure-free days and clinician's and patient's global impression of change [66].

Safety and tolerability

When tried on usual battery of preclinical safety tests, ganaxolone caused only dose-dependent and reversible sedation of experimental animals, while the other findings were unremarkable. Ganaxolone did not show mutagenicity or reproductive toxicity when given to both male and female rats [18].

Although human experiences with ganaxolone are still limited (about 1000 patients received this drug until now), from what was published so far it appears that its safety profile is rather beneficial. No serious adverse reactions that could be ascribed to ganaxolone with certainty were described in published reports, and the adverse reactions that were described were actually the extension of pharmacological action of ganaxolone, and depend on its dose. Since this drug enhances GABA-mediated inhibitory transmission in the central nervous system, it is not surprising that the most frequent adverse effect is somnolence, which affects less than 20% of patients taking ganaxolone. In small children, paradoxical irritability and agitation were noted in less than 10% of cases, what is analogous to similar paradoxical effects observed in the same population with classic sedatives as benzodiazepines. Only in a few cases these adverse effects were severe enough to require withdrawal from the therapy with ganaxolone. While these experiences seem promising, a larger picture about safety profile of ganaxolone will have to wait for completion of new clinical trials.

Regulatory affairs

Ganaxolone is currently tested in the third phase, multicentric clinical trial as add-on therapy for the treatment of partial onset seizures in adults with epilepsy. The results from phase II trials in adults with partial seizures were encouraging, and it is expected that after the third phase studies ganaxolone file will be submitted to the FDA for marketing approval. The Marinus Pharmaceuticals is also trying to obtain clinical evidence for efficacy and safety of ganaxolone in other indications: it is a conducting phase 2 study with ganaxolone as a treatment for behavior disturbances in Fragile X Syndrome and preparing initiation of another phase II study on children of female sex with PCDH19 gene mutation epilepsy, who have deficient GABAergic neurotransmission [67,68].

Summary of the drug characteristics is given in Addendum.

Conclusion

Ganaxolone is an allopregnanolone analogue devoid of hormonal activity which allosterically potentiates inhibitory action of GABA on neurons carrying the GABA-A receptor on their membranes. It prevented seizures in animal models of partial seizures and generalized tonic-clonic seizures, while in the models of the absence of seizures it was either ineffective or prolonged spike wave discharge. Phase I clinical trials pointed to linear pharmacokinetics of ganaxolone, its high protein-binding and metabolism in the liver, and predominant excretion through feces. Ganaxolone was the most efficient as add-on therapy against partial seizures with or without secondary generalization in adult patients, and it is this indication with which the sponsor proceeded to the phase III clinical trials. Although tried in several studies on children suffering from infantile spasms, beneficial effects never reached a statistical significance. Due to its beneficial safety profile (somnolence being the most prominent adverse effect until now) and considerable efficiency in partial onset seizures, it is likely that ganaxolone will be approved as useful adjunct to existing anti-epileptic therapy which could not achieve satisfactory seizure control in adult patients with partial onset seizures.

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Addendum

Drug summary box

Drug name (generic) and route of administration: ganaxolone, oral.
Clinical trial phase (for indication under discussion): phase III.
Pharmacology description/mechanism of action: Ganaxolone binds for unique recognition site on the GABA-A receptor which is different from the binding sites of GABA, benzodiazepines and barbiturates. After binding, ganaxolone potentiates inhibitory action of GABA on neurons which carry the GABA-A receptor.
Indication: drug-resistant, partial onset seizures in adult patients, with or without secondary generalization.
Chemical structure: 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one.
Key trial(s): Phase I: 7 phase I studies conducted on 87 healthy adult male and 9 healthy adult female volunteers [59]; Phase II: Assessment of ganaxolone's anticonvulsant activity against complex partial seizures in adults with epilepsy using a randomized, double-blind, presurgical trial design, and another double-blind, placebo controlled and randomized study conducted with 147 patients suffering from partial onset seizures with or without secondary generalization and refractory to previously used anti-convulsants [61]. Phase III: An ongoing multicenter, randomized, double-blind and placebo-controlled study investigating efficacy and safety of ganaxolone as add-on antiepileptic therapy for adult patients with partial-onset seizures that were not controlled with previous therapy [66].

R E F E R E N C E S

1. *Strzelczyk A, Reese JP, Dodel R, Hamer HM.* Cost of epilepsy: A systematic review. Pharmacoeconomics 2008; 26(6): 463−76.
2. *Jankovic SM, Ignjatovic Ristic D.* Is bioavailability altered in generic versus brand anticonvulsants. Expert Opin Drug Metab Toxicol 2015; 11(3): 329−32.
3. *Jankovic S, Ilickovic I.* The preclinical discovery and development of ezogabine for the treatment of epilepsy. Expert Opin Drug Discov 2013; 8(11): 1429−37.
4. *Jankovic SM, Dostic M.* Choice of antiepileptic drugs for the elderly: possible drug interactions and adverse effects. Expert Opin Drug Metab Toxicol 2012; 8(1): 81−91.
5. *Glauser T, Ben-Menachem E, Bourgeois B, Cnaan A, Chadwick D, Guerreiro C,* et al. ILAE treatment guidelines: evidence-based analysis of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. Epilepsia 2006; 47(7): 1094−120.
6. *Milovanovic JR, Jankovic SM.* Factors influencing carbamazepine pharmacokinetics in children and adults: Population pharmacokinetic analysis. Int J Clin Pharmacol Ther 2011; 49(7): 428−36.
7. *Jankovic SM, Milovanovic JR, Jankovic S.* Factors influencing valproate pharmacokinetics in children and adults. Int J Clin Pharmacol Ther 2010; 48(11): 767−75.
8. *Sánchez-Álvarez JC, Mauri-Llerda JA, Gil-Nagel A, Casas-Fernández C, Salas-Puig J, Lahuerta J*, et al. Consensus-recommended diagnostic and therapeutic guidelines for drug-resistant epilepsy in Spain (Consenso RATE-España). Neurologia 2012; 27(8): 481−90.
9. *Kwan P, Brodie MJ.* Early identification of refractory epilepsy. N Engl J Med 2000; 342(5): 314−9.
10. *Aneja S, Sharma S.* Newer anti-epileptic drugs. Indian Pediatr 2013; 50(11): 1033−40.
11. *Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen HW, Mathern G*, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 2010; 51(6): 1069−77.
12. *Schmidt D, Schachter SC.* Drug treatment of epilepsy in adults. BMJ 2014; 348: g254.
13. *Beyenburg S, Stavem K, Schmidt D.* Placebo-corrected efficacy of modern antiepileptic drugs for refractory epilepsy: systematic review and meta-analysis. Epilepsia 2010; 51(1): 7−26.
14. *Abraham S, Shaju M.* Innovations in epilepsy management: An overview. J Pharm Pharm Sci 2013; 16(4): 564−76.
15. *Luszczki JJ.* Third-generation antiepileptic drugs: mechanisms of action, pharmacokinetics and interactions. Pharmacol Rep 2009; 61(2): 197−216.
16. *Reddy DS.* Neurosteroids: Endogenous role in the human brain and therapeutic potentials. Prog Brain Res 2010; 186: 113−37.
17. *Benjamin M.* Small Biotech Companies Target CNS Disorders. Psychiatric Times [cited 1998 May 1]. Available from: http://www.psychiatrictimes.com/articles/small-biotech-companies-target-cns-disorders
18. *Nohria V, Giller E.* Ganaxolone. Neurotherapeutics 2007; 4(1): 102−5.
19. *Rogawski MA.* Diverse mechanisms of antiepileptic drugs in the development pipeline. Epilepsy Res 2006; 69(3): 273−94.
20. Sigma-Aldrich. G7795 Sigma, Ganaxolone. [accessed 2014 December 28]. Available from: http://www.sigmaaldrich.com/catalog/product/sigma/g7795?lang=en®ion=SX
21. *Shaw K, Zhang M.* Generic Ganaxolone formulations and methods for the making and use thereof. EP1959966A2. 2008.
22. *Puia G, Vicini S, Seeburg PH, Costa E.* Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl- currents. Mol Pharmacol 1991; 39(6): 691−6.
23. *Belelli D, Lambert JJ.* Neurosteroids: Endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 2005; 6(7): 565−75.
24. *Pinna G, Uzunova V, Matsumoto K, Puia G, Mienville JM, Costa E,* et al. Brain allopregnanolone regulates the potency of the GABA(A) receptor agonist muscimol. Neuropharmacology 2000; 39(3): 440−8.
25. *Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ,* et al. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. J Pharmacol Exp Ther 1997; 280(3): 1284−95.
26. *Hosie AM, Wilkins ME, da Silva HM, Smart TG.* Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. Nature 2006; 444(7118): 486−9.
27. *Gasior M, Carter RB, Goldberg SR, Witkin JM.* Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjunction with diazepam. J Pharmacol Exp Ther 1997; 282(2): 543−53.
28. *Gasior M, Ungard JT, Beekman M, Carter RB, Witkin JM.* Acute and chronic effects of the synthetic neuroactive steroid, ganaxolone, against the convulsive and lethal effects of pentylenetetrazol in seizure-kindled mice: comparison with diazepam and valproate. Neuropharmacology 2000; 39(7): 1184−96.
29. *Kaminski RM, Gasior M, Carter RB, Witkin JM.* Protective efficacy of neuroactive steroids against cocaine kindled-seizures in mice. Eur J Pharmacol 2003; 474(2−3): 217−22.
30. *Mares P, Stehlíková M.* Anticonvulsant doses of ganaxolone do not compromise motor performance in immature rats. Neurosci Lett 2010; 469(3): 396−9.
31. *Liptáková S, Velísek L, Velísková J, Moshé SL.* Effect of ganaxolone on flurothyl seizures in developing rats. Epilepsia 2000; 41(7): 788−93.
32. *Yum M, Lee M, Ko T, Velíšek L.* A potential effect of ganaxolone in an animal model of infantile spasms. Epilepsy Res 2014; 108(9): 1492−500.
33. *Kaminski RM, Livingood MR, Rogawski MA.* Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. Epilepsia 2004; 45(7): 864−7.
34. *Reddy DS, Rogawski MA.* Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. J Pharmacol Exp Ther 2000; 295(3): 1241−8.
35. *Reddy DS, Rogawski MA.* Ganaxolone suppression of behavioral and electrographic seizures in the mouse amygdala kindling model. Epilepsy Res 2010; 89(2−3): 254−60.
36. *Reddy DS, Rogawski MA.* Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. J Pharmacol Exp Ther 2000; 294(3): 909−15.
37. *Reddy DS, Rogawski MA.* Neurosteroid replacement therapy for catamenial epilepsy. Neurotherapeutics 2009; 6(2): 392−401.
38. *Snead O.* Ganaxolone, a selective, high-affinity steroid modulator of the?-aminobutyric acid-A receptor, exacerbates seizures in animal models of absence. Ann Neurol 1998; 44(4): 688−91.
39. *Citraro R, Russo E, Di Paola ED, Ibbadu GF, Gratteri S, Marra R,* et al. Effects of some neurosteroids injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy. Neuropharmacology 2006; 50(8): 1059−71.
40. *Beekman M, Ungard JT, Gasior M, Carter RB, Dijkstra D, Goldberg SR,* et al. Reversal of behavioral effects of pentylenetetrazol by

the neuroactive steroid ganaxolone. J Pharmacol Exp Ther 1998; 284(3): 868−77.
41. *Ungard JT, Beekman M, Gasior M, Carter RB, Dijkstra D, Witkin JM.* Modification of behavioral effects of drugs in mice by neuroactive steroids. Psychopharmacology (Berl) 2000; 148(4): 336−43.
42. *Vanover KE, Suruki M, Huber M, Wilent WB, Carter RB.* Neuroactive steroids attenuate cocaine-induced sucrose intake in rats, but not cocaine-induced hyperactivity in mice. Psychopharmacology (Berl) 2000; 149(3): 269−76.
43. *Belelli D, Herd MB.* The contraceptive agent Provera enhances GABA(A) receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids. J Neurosci 2003; 23(31): 10013−20.
44. *Robichaud M, Debonnel G.* Allopregnanolone and ganaxolone increase the firing activity of dorsal raphe nucleus serotonergic neurons in female rats. Int J Neuropsychopharmacol 2006; 9(2): 191−200.
45. *Pinna G, Costa E, Guidotti A.* Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. Psychopharmacology (Berl) 2006; 186(3): 362−72.
46. *Ramaker MJ, Ford MM, Fretwell AM, Finn DA.* Alteration of ethanol drinking in mice via modulation of the GABA(A) receptor with ganaxolone, finasteride, and gaboxadol. Alcohol Clin Exp Res 2011; 35(11): 1994−2007.
47. *Besheer J, Lindsay TG, Buckley TO, Hodge CW, Morrow LA.* Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring p rats. Alcohol Clin Exp Res 2010; 34(12): 2044−52.
48. *Ramaker MJ, Strong MN, Ford MM, Finn DA.* Effect of ganaxolone and THIP on operant and limited-access ethanol self-administration. Neuropharmacology 2012; 63(4): 555−64.
49. *Ramaker MJ, Strong-Kaufman MN, Ford MM, Phillips TJ, Finn DA.* Effect ofnucleus accumbens shell infusions of ganaxolone or gaboxadol on ethanol consumption in mice. Psychopharmacology (Berl) 2015; 232(8): 1415−26.
50. *Ramaker MJ, Ford MM, Phillips TJ, Finn DA.* Differences in the reinstatement ofethanol seeking with ganaxolone and gaboxadol. Neuroscience 2014; 272: 180−7.
51. *Heulens I, Hulst CD, Van DD, De DP, Kooy FR.* Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behav. Brain Res 2012; 229(1): 244−9.
52. *Mellon SH, Gong W, Schonemann MD.* Endogenous and synthetic neurosteroids in treatment of Niemann-Pick Type C disease. Brain Res Rev 2008; 57(2): 410−20.
53. *Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D,* et al. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biol Psychiatry 2006; 60(7): 704−13.
54. *Pinna G, Rasmusson AM.* Ganaxolone improves behavioral deficits in a mousemodel of post-traumatic stress disorder. Front Cell Neurosci 2014; 8: 256.
55. *Pinna G.* Targeting neurosteroidogenesis as therapy for PTSD. Front Pharmacol 2014; 4: 166.
56. *Asiedu MN, Mejia G, Ossipov MK, Malan PT, Kaila K, Price TJ.* Modulation of spinal GABAergic analgesia by inhibition of chloride extrusion capacity in mice. J Pain 2012; 13(6): 546−54.
57. *Paul AM, Branton WG, Walsh JG, Polyak MJ, Lu JQ, Baker GB,* et al. GABA transport and neuroinflammation are coupled in multiple sclerosis: Regulation of the GABA transporter-2 by ganaxolone. Neuroscience 2014; 273: 24−38.
58. *Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White SH.* Progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). Epilepsy Res 2010; 92(2−3): 89−124.
59. *Monaghan EP, Navalta LA, Shum L, Ashbrook DW, Lee DA.* Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. Epilepsia 1997; 38(9): 1026−31.
60. *Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White SH.* Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI). Epilepsy Res 2013; 103(1): 2−30.
61. *Laxer K, Blum D, Abou-Khalil BW, Morrell MJ, Lee DA, Data JL,* et al. Assessment of ganaxolone's anticonvulsant activity using a randomized, double-blind, presurgical trial design. Ganaxolone Presurgical Study Group. Epilepsia 2000; 41(9): 1187−94.
62. *Kerrigan JF, Shields WD, Nelson TY, Bluestone DL, Dodson WE, Bourgeois BF,* et al Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. Epilepsy Res 2000; 42(2−3): 133−9.
63. *Pieribone VA, Tsai J, Soufflet C, Rey E, Shaw K, Giller E,* et al. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. Epilepsia 2007; 48(10): 1870−4.
64. *McAuley JW, Reeves Al, Flyak J, Monaghan EP, Data J.* A pilot study of the neurosteroid ganaxolone in catamenial epilepsy: Clinical experience in two patients. Epilepsia 2001; 42(Suppl 7): 85.
65. *Reddy DS, Rogawski MA.* Neurosteroids: Endogenous Regulators of SeizureSusceptibility and Role in the Treatment of Epilepsy. In: *Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV*, editors. Jasper's Basic Mechanisms of the Epilepsies. 4th ed. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
66. ClinicalTrials.gov. Phase 3 Study of Adjunctive Ganaxolone in Adults With Drug-resistant Partial Onset Seizures, With Long-term Open-label Extension. Identifier: NCT01963208. Available from: https://clinicaltrials.gov/ct2/show/NCT01963208?term=ganaxolone&rank=4
67. Marinus pharmaceuticals provides business update and reports third quarter 2014 financial results. 2014. Radnor, pa., nov. 13, (globe newswire). Available from: http://ir.marinuspharma.com/releasedetail.cfm?releaseid=882728
68. *Sánchez-Alvarez JC, Serrano-Castro P, Cañadillas-Hidalgo F.* Refractory epilepsy in adults. Rev Neurol 2002; 35(10): 931−53.

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Extraskeletal activity of vitamin D and a potential association with diabetes mellitus

Vanskeletna aktivnost vitamina D i njegova potencijalna udruženost sa šećernom bolesti

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Key words:
diabetes mellitus; vitamin D; vitamin D deficiency; risk assessment; dietary supplements.

Ključne reči:
dijabetes melitus; vitamin D; vitamin D, nedostatak; rizik, procena; ishrana, dopune.

Introduction

For decades, vitamin D has been characterised with its important role in regulating the serum levels of calcium and phosphorus, as well as in maintenance of bone and mineral metabolism. In addition to its classical action, an increasing amount of available data suggests a possible involvement of vitamin D activity in many other pathophysiological fields, such as inflection of immune response and inflammation, cell proliferation or gene expression [1]. Many experimental and clinical data indicate the impact of vitamin D on different steps in onset and development of diabetes, representing this way its potential beneficial influence on morbidity, glycemic control and the incidence of chronic complications [2].

Metabolism of vitamin D

Vitamin D is a group of sterols with a hormone-like activity, that can be consumed from food or synthesized in the skin [3]. There are two inactive forms of vitamin D: cholecalciferol (also called vitamin D3), that comes from foods of animal origin, and ergocalciferol (also called vitamin D2) which is of plant origin. When the skin is exposed to solar ultraviolet B radiation, cholecalciferol could also be rapidly converted from its precursor, called 7-dehydrocholesterol. After entering into blood stream from guts or skin, these inactive forms of vitamin D, are transported to the liver binded to the vitamin D-binding protein (VDBP). Next step in the activation process, is the hydroxylation at C-25 *via* vitamin D-hydroxylase enzyme, forming 25-hydroxyvitamin D_3 [25 (OH) D, also called calcidiol]. This is the major form of storage and detection of vitamin D. Almost all calcidiol is bound to circulating VDBP and transported to kidneys. At the level of the proximal renal tubul, this metabolite is further hydroxylated by the 1α-hydroxylase enzyme forming 1α,25 dihydroxyvitamin D_3 [1α,25 $(OH)_2D$, also called calcitriol]. This is the active form of vitamin D [3, 4].

Apart from the kidneys, many other tissues have the ability to convert calcidiol into calcitriol, since the enzyme 1α-hydroxylase has been observed in placenta, breasts, colon, prostate, macrophages or monocytes. However, in humans, this extrarenal sources of calcitriol only contribute significantly to circulating levels of this active vitamin D form during pregnancy, in chronic renal failure, in sarcoidosis, tuberculosis, granulomatous diseases and rheumatoid arthritis [5].

The production of calcitriol is regulated by serum calcium and phosphorus levels, plasma parathyroid hormone (PTH) levels and fibroblast growth factor 23 (FGF23). Most of the biological activities of calcitriol are mediated by a high-affinity receptor that acts as a ligand-activated transcription factor. This cytosolic/nuclear vitamin D receptor (VDR) is a transcriptional activator of many genes and is widely distributed in more than 38 types of tissues, controlling bone metabolism, inflammation, oxidative damage or chronic diseases [6]. This distribution becomes especially important in understanding of extraskeletal effects of vitamin

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D. This is due, not only the ability of different tissues to synthesized calcitriol, but also because of widespread distribution of the specific VDR that mediated vitamin D action [4].

Vitamin D effects on the immune process and type 1 diabetes

Type 1 diabetes mellitus is chronic progressive autoimmune disease characterized by mononuclear cell infiltration, dominantly by interleukin (IL)-12-dependent T helper type 1 (Th1) cells of the pancreatic islets, with subsequent β-cells destruction and decreased insulin secretion. After 70−90% of β-cells are destroyed, available insulin is no longer adequate to maintain normal blood glucose level and diabetes may be diagnosed. Thus, an autoimmune destruction process plays a central role in the development of type 1 diabetes, and is mediated by the subjects own genetic susceptibility and by non-genetic factors. Vitamin D deficiency is one of the non-genetic factors that could be associated with an increased risk of developing of autoimmune diabetes. In favor of that, the incidence of type 1 diabetes follows a geographical pattern, with reported association between this type of diabetes mellitus and vitamin D status [7]. Vitamin D has a protective effect on the pancreatic β-cells. VDR is detected in almost all cells of the immune system, especially antigen-presenting cells (dendritic cells and macrophages) and activated T-lymphocytes. These cells are also able to synthesize and secrete calcitriol since they possess the enzyme 1α-hydroxylase. Although multiple, the main effect of calcitriol on the immune system is leading to the generation of tolerance and anergy, rather than immune activation [4]. For instance, at the level of dendritic cells, calcitriol inhibits the surface expression of major histocompatibility complex (MHC) class II-complexed antigen. Thus, these cells do not mature at a subsequent exposure to an antigen, but become tolerogenic. IL-12, the major cytokine managing the immune system towards Th1 development, is almost totally inhibited in the presence of calcitriol. The same goes to several other inflammatory T cell cytokines, such as IL-2, interferon-gamma (IFN-γ), and tumor necrosis factor-alfa (TNFα), while the production of anti-inflammatory cytokines like IL-4 or IL-10 is stimulated. These immunomodulatory effects of calcitriol can lead to the protection of target tissues such as β-cells in type 1 diabetes [8].

Treatment of non-obese diabetic (NOD) mouse, which represents an animal model for human type 1 diabetes, with calcitriol analog, can prevent dendritic cells maturation, decreased IL-12 and IFNγ production and arrests Th1 cell infiltration. These processes lead to the inhibition of *insulinitis* and slow down the progression of type 1 diabetes [9]. Clinical type 1 diabetes can also be prevented in animal models, if calcitriol analogues are administered to NOD mice when the autoimmune disease is already active [10].

Simultaneously to experimental data, there are some clinical studies confirming protective immunological effect of vitamin D supplementation. In 38 patients with new-onset type 1 diabetes, cholecalciferol supplementation led to increased level of regulatory T-cells, serum IL-10 levels and significant increase of monocyte chemoattractant protein-1 (MCH-1) levels. This protective effect might contribute to preservation of residual C-peptide secretion obtained in this study [11]. Several observational clinical studies raised the possibility that vitamin D intake during early life, may prevent the development of type 1 diabetes [12, 13]. The first prospective study of cholecalciferol supplementation in infants was conducted in Northern Finland [14]. This study provides the evidence that high doses of vitamin D [2,000 international units (IU) daily or more], during the first year of life, may reduce the risk for type 1 diabetes, at least in the parts of the world where yearly sunlight is limited. Still, some other studies did not confirm this association [15, 16].

Conflicting results were also observed in studies related to vitamin D deficiency in the fetal period and risk of type 1 diabetes. In a Swedish cohort study, a weak inverse association was observed between maternal vitamin D supplementation and the appearance of diabetes-associated autoantibodies at the age of 1 year, but not at 2.5 years [17]. Maternal intake of vitamin D from food, but not from supplements during pregnancy was associated with a decreased risk of early islet autoimmunity appearance in the offspring [18]. Lower risk of type 1 diabetes in the offspring was observed in women using cod liver oil during pregnancy [19]. On the other hand, Marjamäki et al. [20], found no association between the maternal intake of vitamin D, either from food or from supplements, with the risk of advanced β-cell autoimmunity and type 1 diabetes in offsprings [20]. Similarly, measuring of calcidiol concentrations during the first trimester of pregnancy, showed no difference between mothers whose children later on developed type 1 diabetes, and mothers of "non diabetic" healthy children [21].

Vitamin D effects on insulin secretion, insulin resistance and glucose control in type 2 diabetes

Potential influence of vitamin D on glucose handling is based on experimental data, that include the expression of 1α-hydroxylase enzyme and the presence of VDRs on pancreatic β-cells, as well as the presence of VDR on skeletal muscle. Calcitriol also stimulates the expresion of insulin receptor, activates peroxisome proliferator-activated receptor gamma (PPRγ) and enhances insulin-mediated glucose transport *in vitro* [22]. *In vitro*, calcitriol induces the biosynthesis of insulin in rat pancreatic islet cells and improves glucose uptake in cultured myocytes in a dose-dependent manner. Moreover, calcitriol protects pancreatic ß-cells from immune attacks, directly and indirectly by enhancing dendritic cell maturation, T-cells proliferation and macrophage differentiation [23].

Possible mechanisms od vitamin D activity on insulin secretion or sensitivity include: its effect on intracellular calcium levels, diminishing the expresion of proinflammatory citokines involved in insulin resistance such as IL-1, IL-6 and down regulation of nuclear factor kappa B activity [24]. Obesity is commonly associated with vitamin D deficiency, due to the capacity of adipocytes to store calcidiol, making it biologically unavailable [25]. On the other hand, decreased amount of serum calcidiol, calcitriol and raised PTH, can le-

ad to increased intracellular calcium in adipose tissue, which can in turn stimulate lipogenesis, increasing this way the risk of metabolic syndrome and type 2 diabetes [26].

In spite of the mentioned biological data reffering a potential influence of vitamin D to insulin secretion and action, results of conducted clinical studies are pretty inconsistent.

In healthy volunteers, calcidiol concentrations showed a positive relation to insulin sensitivity, and a negative effect on ß-cell function using the hyperglycemic clamp tehnique [27]. In the group of 157 individuals with prediabetes, serum calcidiol levels had a significant inverse correlation with insulin resistance measured by homeostasis model assessment (HOMA2) index, and a positive correlation with insulin sensitivity [28]. A significant inverse association has been reported between calcidiol levels and oral glucose tolerance test (OGTT) − induced insulin secretion in elderly [29]. Similar results regarding positive relationship between serum calcidiol and insulin sensitivity were reported by some other autors [30–32].

On the other hand, prospective cross sectional study conducted on type 2 diabetics enrolled from the urban Indian population, showed no association of serum calcidiol deficiency, on metabolic control or insulin restistance measured by HOMA of insulin resistance (HOMA IR) [33]. Measuring insulin sensitivity with the euglycemic-hyperinsulinemic clamp tehnique in morbidly obese Cavcasian women before and after bariatric surgery, showed no possitive correlation between D vitamin leveles and periferal glucose uptake [34]. The same goes to some other populations [35, 36]. The reasons for such conflicting results might originate from different optimal serum concentrations of calcidiol for different ethnicity, dissimilar methodological approach in measuring of insulin sensitivity, or relativly small sample size in mentioned studies.

The effect of vitamin D supplementation on glucose homeostasis have been studied in many researches. Oral weekly supplemental vitamin D (dose of 50,000 IU) given for two months, significantly decreased serum fasting plasma glycemia and insulin resistance (HOMA IR) in a group of type 2 diabetic patients [37]. A similar association between vitamin D supplementation and insulin sensitivity and fasting glucose levels was obtained in some other studies [27, 38]. Insulin sensitivity was also improved in nondiabetic patients with monthly suplementation with 120,000 IU of vitamin D [39]. In contrast to these results, cholecalciferol supplementation during 6 months (20,000 IU twice weekly) to apparently healthy subjects with insufficient serum calcidiol levels, did not improve insulin secretion or sensitivity using hyperglycemic clamp technique [40]. Some other studies using HOMA β as insulin secretory outcome, also did not observe significant changes in insulin secretion after cholecalciferol suplementation [41, 42]. Disperities in mentioned studies could be partly explained by using HOMA IR instead of more sensitive clamp tehniques in some studies. Supplementation of vitamin D-sufficient populations could be an additional factor; using calcitriol or/and vitamin D analogues instead of oraly supplemental cholecalciferol, could also contribute to result unsteadiness.

As for prospective studies, it seems that they provide the potentially strongest evidence for the relationship of basal plasma calcidiol values and subsequent glycemic control. In the group of 524 non-diabetic persons, baseline values of calcidiol were inversely associated with a 10-year risk of fasting hyperglycemia, insulin resistance and metabolic syndrome [43]. Similarly, a Finnish cohort study showed an inverse relationship between baseline calcidiol levels and a 17-year risk of type 2 diabetes [44]. Finally, meta analysis of 21 prospective studies, that included nearly 5,000 incident cases of type 2 diabetes and 76,220 nondiabetic controls, confirms a significantly inverse association between calcidiol levels and the incidence of type 2 diabetes. This association did not differ markedly by sex, study size or calcidiol assay method [45].

Vitamin D and chronic complications of diabetes

Vitamin D deficiency rate reported to be higher among patients with both types of diabetes [46, 47]. In contrast to numerous evidence that hypovitaminosis D is associated with higher prevalence of type 1 and type 2 diabetes, data of mutual connections between vitamin D status and chronic diabetic complications are scarce. Vitamin D have several immunomodulatory effects, such as inhibition of the renin-angiotensin system and reduction of inflammatory activity, that can be correlated with pro-inflammatory condition that is typical of diabetes.

Some observational studies in small samples, noticed a significant association between lower calcidiol levels and risk of all-cause or cardiovascular mortality in patients with type 2 and type 1 diabetes [48, 49]. Currently the largest, observational study on 472 men and 245 women with type 2 diabetes confirmed an independent relationship between low calcidiol levels and all-cause mortality, but only in men. This relationship was still significant when two other risk markers for mortality (pulse wave velocity and carotid intima-media thickness) were added to analysis, suggesting the posibility that vitamin D can be used as a surrogate marker of risk for mortality in male type 2 diabetic patients. These results also suggest that potential non-skeletal effects of vitamin D is gender-dependent [50].

In the cross-sectional study on 715 type 2 diabetic patients, there was a significant inverse association between circulating vitamin D levels and the presence of retinopathy and/or nephropathy [46]. This inverse and independent relationship was maintained even when the analysis was confined only to patients with glomerular filtration rate above 60 mL/min/1.73 cm^2. Vitamin D deficiency was associated with increased prevalence of retinopathy in young people with type 1 diabetes and in patients with type 2 diabetes, even after adjustment for potential confounders [51, 52]. Another study on 1,520 type 2 diabetic patients showed that vitamin deficiency is an independent risk factor for retinopathy [53]. The same study showed that the prevalence of sight threatening diabetic retinopathy doubles when the serum vitamin D levels are less than 15,57 ng/mL. In a retrospective study on 557 type 2 diabetic patients, vitamin D deficiency was lower in subjects with more severe diabetic microvascular compli-

cations [54]. On the other hand, some other autors did not confirm this relationship. In the prospective observational study on a cohort of type 1 diabetic patients, severe vitamin D deficiency, independently predict all cause mortality, but not the development of retinopathy or nephropathy [49].

As for neuropathy, one meta-analysis showed that vitamin D deficiency was significantly associated with increased risk of diabetic neuropathy in patients with type 2 diabetes. Vitamin D insufficiency was also associated with reduced parasympathetic function in type 2 diabetes, while the onset of neuropathy can be delayed by vitamin D treatment in type 1 diabetic patients [55−57].

A potential protective effect of vitamin D on the onset and progression of diabetic complications, originates from *in vitro* and *in vivo* experimental studies. Calcitriol is a powerful inhibitor of angiogenesis in mouse models of retinopathy and *in vitro* studies on retinal endothelial cells [58]. High serum calcitriol levels were associated with reduced angiogenesis in transgenic retinoblastoma model and in ischemic retinopathy in mice [59].

There are also some experimental evidence that VDR is a modulator of glomerular injury. Calcitriol decreases the glomerulosclerosis index and urinary albumin excretion (UAE) in animal models, while the combination of VDR-activator and an angiotensin-converting ezyme (ACE) inhibitor protected mice from developing diabetic nephropathy (DN). Vitamin D receptor agonists also reduce expresion of inflammatory mediators by monocyte and T-cells, promote survival of podocytes by preventing their apoptosis. Vitamin D is a potent negative endocrine regulator of the renin-angiotensin system (RAS) and predominantly works as a suppressor of renin biosynthesis. Calcitriol also suppresses hyperglycemia-induced activation of the RAS and transforming growth factor beta (TGFβ) in mesangial and juxtaglomerular cells, acting this way on one of the main mechanisam of renal injury in diabetes [60].

As for clinical studies, it is well-known that patients with chronic kidney disease (CKD), regardless of etiology, have active vitamine D deficiency. Serum calcidiol levels begin to decrease in stages 2 CKD, and its deficiency is prevalent in all subsequent stages of CKD. This could be influenced by the loss of VDBP in urine, ineffective synthesis in skin or reduced nutritional intake [61] . In a prospective follow up study on 168 patients with CKD, calcidiol levels were indipendent inverse predictor of disease progression and death, in patients with stages 2−5 of CKD [62]. This association was the strongest among patients with DN. In the prospective study on 103 patients with type 2 diabetes and DN, vitamin D deficiency was associated with accelerated progression of CKD after a median follow-up of 32 months, even though all patients have been received optimal RAS blockade [63]. Finally, the first clinical study that clearly suggests potential renoprotective effect of vitamin D supplementation was the study of Kim et al. [64]. In the group of 63 type 2 diabetic patients with nephropathy, treatment with oral cholecalciferol for 4 months, significantly decreased UAE and urinary TGF-β1 excretion, which represents the prinicipal mediator of onset and progression of diabetic kidney disease.

Vitamin D supplementation: current recommendations

The most accurate way to determine vitamin D status is measuring of calcidiol. Vitamin D deficiency in adults, is defined as a serum calcidiol level of less than 50 nmol/L (20 ng/mL), while insufficiency is defined as a serum calcidiol level of 50 to 75 nmol/L (20 to 30 ng/mL) [65].

A possible explanation for the actual widespread vitamin D deficiency is the lack of sunlight exposure, since the humans typically obtain 90 percent of vitamin D from skin synthesis. It is thought that 5 to 30 min of sun exposure of face, arms, back or legs, at least twice *per* week is usually adequate for vitamin D synthesis. Other factors contributing to vitamin D deficiency are increased use of sunscreen, pollution, dark or aging skin, seasons, latitude, sedentary lifestyle, obesity and use of some medications like glucocorticoids or anticonvulsants, which can increase catabolism of vitamin D. Furthermore, dermatologists caution against direct sun exposure to avoid risks of skin damage or skin cancer; so the useful alternative is supplemental vitamin D [66].

There is still no universally accepted standard regimen for overal correction of vitamin D deficiency, including diabetic state. Thus, there are no specific recommendations for vitamin D supplementations for diabetic patients or pregnant diabetic women, and the treatment strategy is the same as for the general population.

Current referrals are formed as a result of the previously mentioned clinical studies, taking into account the tolerable upper intake level. It is also important to point out, that the vitamin D supplementation is relatively safe, and that the toxicity have been observed only in patients taking more than 40,000 IU/daily [67].

In contrast to the World Health Organisation (WHO), which has not change its referrals from 2004, the Food and Nutrition Board of the American Institute of Medicine notified its new recommendations for vitamin D supplementation in 2010 [68, 69]. The later organisation recommends daily intake of 600 IU of vitamin D for persons aged 9−70 years; 800 IU daily intake for individuals over 70 years and 600 IU daily intake for pregnancy and lactation. Infants are recommended to intake 400 IU per day during the first 12 months, and 600 IU for everyone older than one year of age [69]. The American Academy of Pediatrics shares the same recommendations [70]. This organisation consider that the safe upper limit for vitamin D is 1,000 to 1,500 IU daily for infants; 2,500 to 3,000 IU daily for children between age of 1 to 8 years and 4,000 IU daily for children over 9 years of age, adults and pregnant women. According to the Endocrine Society Clinical Practice Guidelines, daily regimen for pregnant women includes taking products that contain at least 1,000 IU of vitamin D. For lactating women it is recomeneded to take 1,400−1,500 IU vitamin D every day, and to satisfy infant's requirement, they may need 4,000−6,000 IU/daily, if they choose not to give the infant a vitamin D supplements. They also suggets that obese children and adults sho-

uld be taken at least two or three times more vitamin D for age group to satisfy their body requirement [71]. Unlike these organisations, WHO consider that there is no indications for vitamin D supplementation during pregnancy [72]. Some autors claim that there may be no preventive effectiveness of early supplementation with 400 IU/daily of vitamin D or less, while higher doses of 2,000 IU/ daily could provide stronger protective effect against type 1 diabetes [67]. It should be also provided that all infants and children receive between 200 and 1,000 IU of suplemental vitamin D daily, especially if they have limited sun exposure, exclusively breastfed or, are at increased risk of type 1 diabetes. Guided by the results of previous studies, some other autors assume that vitamin D dose needs to be high enough, above 2,000 IU/daily, to raise blood calcidiol levels above 80 nmol/L, because diabetes risk is the lowest at this level [73].

As for patients with DN and CKD, in the majority of guidelines, vitamin D substitution strategies are based on serum calcidiol, calcium and PTH levels, mainly in order to reduce risk for secondary hyperparathyroidism. According to Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, ergocalciferol should be used in CKD stages 3 and 4, when serum level of calcidiol is less than 30 ng/mL [74]. For those with high PTH and calcidiol level more than 30 ng/mL in CKD stages 3 and 4, substitution is recommended with active oral steroids (calcidiol, calcitriol or calcitriol analogues such as paricalcitol). Potential nephroprotective effect of cholecalciferol substitution on diabetic kidney disease is still under investigation, and therefore, has not been included in official recommendations.

Conclusion

Although promising, the data on the association between vitamin D and both types of diabetes are still inconclusive. There is also no clearly defined answer to what are the optimal concentrations of vitamin D for optimal glucose maintenance, or whether vitamin D supplementation may provide better clinical course of diabetes and reduce risk for diabetic complications. In order to resolve this problem, large randomized controlled clinical trials of the effect of vitamin D supplementation on glycemic control and diabetic risk are required, providing this way a simple and inexpensive additional assistance in prevention of diabetes mellitus all over the world.

R E F E R E N C E S

1. *Guo J, Liu Z.* Multiple effects of vitamin D. Chin Med J 2013; 126(15): 2978−83.
2. *Alaverz J, Ashraf A.* Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010; 2010: 351385.
3. *Li J, Xiao B, Xiang Y.* Immune function of vitamin D in type 1 diabetes mellitus. IJBM 2014; 4(2): 67−71.
4. *Mathieu C, Badenhoop K.* Vitamin D and type 1 diabetes mellitus: State of the art. Trends Endocrinol Metab 2005; 16(6): 261−6.
5. *Valdivielso JM, Fernandez E.* Vitamin D receptor polymorphisms and diseases. Clin Chim Acta 2006; 371(1−2): 1−12.
6. *Henry HL.* Regulation of vitamin D metabolism. Best Pract Res Clin Endocrinol Metab 2011; 25(4): 531−41.
7. *Gillespie KM.* Type 1 diabetes: Pathogenesis and prevention. CMAJ 2006; 175(2): 165−70.
8. *Bikle DD.* Vitamin D regulation of immune function. Vitam Horm 2011; 86: 1−11.
9. *Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L.* A 1α, 25-dihydroxyvitaminD3 analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 2001; 51(5): 1367−74.
10. *Casteels KM, Mathieu C, Waer M, Valckx D, Overbergh L, Laureys JM*, et al. Prevention of type I diabetes in nonobese diabetic mice by late intervention with nonhypercalcemic analogs of 1,25-dihydroxyvitamin D3 in combination with a short induction course of cyclosporin A. Endocrinology 1998; 139(1): 95−102.
11. *Gabbay MA, Sato MN, Finazzo C, Duarte AJ, Dib SA.* Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual β-cell function in new-onset type 1 diabetes mellitus. Arch Pediatr Adolesc Med 2012; 166(7): 601−7.
12. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. Diabetologia 1999; 42(1): 51−4.
13. *Stene LC, Joner G.* Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: A large, population-based, case-control study. Am J Clin Nutr 2003; 78(6): 1128−34.
14. *Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM.* Intake of vitamin D and risk of type 1 diabetes: A birth-cohort study. Lancet 2001; 358(9292): 1500−3.
15. *Visalli N, Sebastiani L, Adorisio E, Conte A, De Cicco A, D'Elia R*, et al. Environmental risk factors for type 1 diabetes in Rome and province. Arch Dis Child 2003; 88(8): 695−8.
16. *Simpson M, Brady H, Yin X, Seifert J, Barriga K, Hoffman M*, et al. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia 2011; 54(11): 2779−88.
17. *Brekke HK, Ludvigsson J.* Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. Pediatr Diabetes 2007; 8(1): 11−4.
18. *Fronczak CM, Barón AE, Chase PH, Ross C, Brady HL, Hoffman M*, et al. In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care 2003; 26(12): 3237−42.
19. *Stene LC, Ulriksen J, Magnus P, Joner G.* Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 2000; 43(9): 1093−8.
20. *Marjamäki L, Niinistö S, Kenward MG, Uusitalo L, Uusitalo U, Ovaskainen ML*, et al. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia 2010; 53(8): 1599−607.
21. *Miettinen ME, Reinert L, Kinnunen L, Harjutsalo V, Koskela P, Surcel HM,* et al. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. Diabetologia 2012; 55(5): 1291−4.
22. *Matsuda S, Kitagishi Y.* Peroxisome proliferator-activated receptor and vitamin D receptor signaling pathways in cancer cells. Cancer 2013; 5(4): 1261−70.
23. *Bourlon PM, Billaudel B, Faure-Dussert A.* Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. J Endocrinol 1999; 160(1): 87−95.

24. *Maestro B, Dávila N, Carranza CM, Calle C.* Identification of a Vitamin D response element in the human insulin receptor gene promoter. J Steroid Biochem Mol Biol 2003; 84(2−3): 223−30.
25. *Moreira T, Hamadeh M.* The role od vitamin D deficiency in the pathogenesis of type 2 diabetes. E Spen Eur E J Clin Nutr Metab 2010; 5(4): e155−65.
26. *Takiüshi T, Gysemans C, Bouillon R, Mathieu C.* Vitamin D and diabetes. Rheum Dis Clin North Am 2012; 38(1): 179−206.
27. *Chiu KC, Chu A, Go VL, Saad MF.* Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004; 79(5): 820−5.
28. *Dutta D, Maisnam I, Shrivastava A, Sinha A, Ghosh S, Mukhopadhyay P,* et al. Serum vitamin-D predicts insulin resistance in individuals with prediabetes. Indian J Med Res 2013; 138(6): 853−60.
29. *Baynes KC, Boucher BJ, Feskens EJ, Kromhout D.* Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia 1997; 40(3): 344−7.
30. *Hyppönen E, Power C.* Vitamin D status and glucose homeostasis in the 1958 British birth cohort: The role of obesity. Diabetes Care 2006; 29(10): 2244−6.
31. *Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H,* et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes Care 2009; 32(7): 1278−83.
32. *Chonchol M, Scragg R.* 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. Kidney Int 2007; 71(2): 134−9.
33. *Sheth J, Shah A, Sheth F, Trivedi S, Lele M, Shah M,* et al. Does vitamin D play a significant role in type 2 diabetes. BMC Endocr Disord 2015; 15: 5.
34. *Manco M, Calvani M, Nanni G, Greco AV, Iaconelli A, Gasbarrini G,* et al. Low 25-hydroxyvitamin D does not affect insulin sensitivity in obesity after bariatric surgery. Obes Res 2005; 13(10): 1692−700.
35. *Alemzadeh R, Kichler J, Babar G, Calhoun M.* Hypovitaminosis D in obese children and adolescents: Relationship with adiposity, insulin sensitivity, ethnicity, and season. Metab Clin Exp 2008; 57(2): 183−91.
36. *McGill A, Stewart JM, Lithander FE, Strik CM, Poppitt SD.* Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J 2008; 7: 4.
37. *Talaei A, Mohamadi M, Adgi Z.* The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metabol Syndr 2013; 5: 8.
38. *von Hurst PR, Stonehouse W, Coad J.* Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. Br J Nutr 2010; 103(4): 549−55.
39. *Pittas AG, Harris SS, Stark PC, Dawson-Hughes B.* The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007; 30(4): 980–6.
40. *Grimnes G, Figenschau Y, Almås B, Jorde R.* Vitamin D, insulin secretion, sensitivity, and lipids: Results from a case-control study and a randomized controlled trial using hyperglycemic clamp technique. Diabetes 2011; 60(11): 2748−57.
41. *Jorde R, Figenschau Y.* Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. Eur J Nutr 2009; 48(6): 349−54.
42. *Nagpal J, Pande JN, Bhartia A.* A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. Diabet Med 2009; 26(1): 19−27.
43. *Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ.* Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: The Medical Research Council Ely Prospective Study 1990-2000. Diabetes 2008; 57(10): 2619−25.
44. *Mattila C, Knekt P, Männistö S, Rissanen H, Laaksonen MA, Montonen J,* et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care 2007; 30(10): 2569−70.
45. *Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE,* et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: A meta-analysis of prospective studies. Diabetes Care 2013; 36(5): 1422−8.
46. *Zoppini G, Galletti A, Targher G, Brangani C, Pichiri I, Trombetta M,* et al. Lower levels of 25-hydroxyvitamin D3 are associated with a higher prevalence of microvascular complications in patients with type 2 diabetes. BMJ Open Diab Res Care 2015; 3(1): e000058.
47. *Svoren BM, Volkening LK, Wood JR, Laffel LM.* Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr 2009; 154(1): 132−4.
48. *Joergensen C, Gall M, Schmedes A, Tarnow L, Parving H, Rossing P.* Vitamin D levels and mortality in type 2 diabetes. Diabetes Care 2010; 33(10): 2238−43.
49. *Joergensen C, Hovind P, Schmedes A, Parving H, Rossing P.* Vitamin D levels, microvascular complications, and mortality in type 1 diabetes. Diabetes Care 2011; 34(5): 1081−5.
50. *Jennersjo P, Guldbrand H, Bjorne S, Lanne T, Fredrikson M, Lindstrom T,* et al. A prospective observational study of all-cause mortality in relation to serum 25-OH vitamin D 3 and parathyroid hormone levels in patients with type 2 diabetes. Diabetol Metab Syndr 2015; 7: 53.
51. *Kaur H, Donaghue KC, Chan AK, Benitez-Aguirre P, Hing S, Lloyd M,* et al. Vitamin D deficiency is associated with retinopathy in children and adolescents with type 1 diabetes. Diabetes Care 2011; 34(6): 1400−2.
52. *Ahmadieh H, Azar S, Lakkis N, Arabi A.* Hypovitaminosis D in patients with type 2 diabetes mellitus: A relation to disease control and complications. ISRN Endocrinol 2013; 2013: 641098.
53. *He R, Shen J, Liu F, Zeng H, Li L, Yu H,* et al. Vitamin D deficiency increases the risk of retinopathy in Chinese patients with type 2 diabetes. Diabet Med 2014; 31(12): 1657−64.
54. *Usluogullar CA, Balkan F, Caner S, Ucler R, Kaya C, Ersoy R,* et al. The relationship between microvascular complications and vitamin D deficiency in tyšpe 2 diabetes mellitus. BMC Endocr Disord 2015; 15: 33.
55. *Lv WS, Zhao WJ, Gong SL, Fang DD, Wang B, Fu ZJ,* et al. Serum 25-hydroxyvitamin D levels and peripheral neuropathy in patients with type 2 diabetes: A systematic review and meta-analysis. J Endocrinol Invest 2015; 38(5): 513−8.
56. *Maser RE, Lenhard JM, Pohlig RT.* Vitamin D Insufficiency is Associated with Reduced Parasympathetic Nerve Fiber Function in Type 2 Diabetes. Endocr Pract 2015; 21(2): 174−81.
57. *Shehab D, Al-Jarallah K, Mojiminiyi OA, Al Mohamedy H, Abdella NA.* Does Vitamin D deficiency play a role in peripheral neuropathy in Type 2 diabetes. Diabet Med 2012; 29(1): 43−9.
58. *Albert DM, Scheef EA, Wang S, Mehraein F, Darjatmoko SR, Sorenson CM,* et al. Calcitriol is a potent inhibitor of retinal neovascularization. Invest Ophthalmol Vis Sci 2007; 48(5): 2327−34.
59. *Shokravi MT, Marcus DM, Alroy J, Egan K, Saornil MA, Albert DM.* Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma. Invest Ophtalmol Vis Sci 1995; 36(1): 83−7.
60. *Sanchez-Niño M, Bozic M, Córdoba-Lanús E, Valcheva P, Gracia O, Ibarz M,* et al. Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy. Am J Physiol Renal Physiol 2012; 302(6): F647−57.

61. *Agarwal R.* Vitamin D, proteinuria, diabetic nephropathy, and progression of CKD. Clin J Am Soc Nephrol 2009; 4(9): 1523−8.
62. *Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P*, et al. Vitamin D levels and patient outcome in chronic kidney disease. Kidney Int 2009; 75(1): 88−95.
63. *Fernandez-Juarez G, Luno J, Barrio V, de Vinuesa GS, Praga M, Goicoechea M*, et al. 25(OH) Vitamin D lelevs and renal disease progression in patients with type 2 diabetic nephropathy and blockade of the renin-angiotensin sysem. Clin J Am Soc Nephrol 2013; 8(11): 1870−6.
64. *Kim MJ, Frankel AH, Donaldson M, Darch SJ, Pusey CD, Hill PD*, et al. Oral cholecalciferol decreases albuminuria and urinary TGF-β1 in patients with type 2 diabetic nephropathy on established renin-angiotensin-aldosterone system inhibition. Kidney Int 2011; 80(8): 851−60.
65. *Bordelon P, Ghetu MV, Langan RC.* Recognition and management of vitamin D deficiency. Am Fam Physician 2009; 80(8): 841−6.
66. *Marti T, Campbel RK.* Vitamin D and diabetes. Diabet Spec 2011; 24(2): 113−8.
67. *Harris SS.* Vitamin D in type 1 diabetes prevention. J Nutr 2005; 135(2): 323−5.
68. WHO Guideline. Vitamin and mineral requirements in human nutrition. Geneva: World Health Organisation and Food and Agriculture Organisation of the United Nations; 2004.
69. *Institute of Medicine Food and Nutrition Board.* Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. Washington, D.C: National Academy Press; 2010.
70. American Academy of Pediatrics. Committy on nutrition. In: Pediatric Nutrition Handbook. 6th ed. Elk Grove Village, IL: American Acadamey of Pediatrics; 2008.
71. *Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP,* et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96(7): 1911−30.
72. *WHO.* Guideline: Vitamin D supplementation in pregnant women. Geneva: World Health Organization; 2012.
73. *Scragg R.* Vitamin D and type 2 diabetes: Are we ready for a prevention trial. Diabetes 2008; 57(10): 2565−6.
74. *National Kidney Foundation.* K/DOQI clinical practice guidelines for bone metabolism and diseasein chronic kidney disease. Am J Kidney Dis 2003; 42(4 Suppl 3): S1−201.

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Intraosseous focal venous malformation of the mandibular body: cone beam computed tomography planning followed by piezoelectric knife resection and free bone graft reconstruction

Intraosealna fokalna venska malformacija tela donje vilice: planiranje piezoelektrične resekcije pomoću kompjuterizovane tomografije konusnim zrakom i rekonstrukcija slobodnim koštanim transplantatom

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Abstract

Introduction. Intraosseous vascular malformation could be life-threatening due to uncontrolled hemorrhage after tooth extraction. According to biological behavior of this lesion, adequate diagnostic and treatment strategies are necessary in order to avoid possible complications. We reported cone beam computed tomography (CBCT) planning of an urgent *en bloc* resection of an intraosseous venous malformation by piezoelectric knife. **Case report.** A 55-year-old man was submitted to CBCT planning followed by piezoelectric knife resection of an intraosseous focal venous malformation of the mandibular body. Immediate reconstruction of the defect using iliac bone free graft was performed. The surgical treatment was uneventful and a 2-year follow-up revealed no signs of recurrence. **Conclusion.** Piezoelectric knife could provide precise, safe and bloodless procedure which is especially important in this pathology. Advantages of this technique are: lower risk of damaging soft tissue structures, precise osteotomy and bloodless surgery. Moreover, using piezosurgery bone knife, blood transfusion and blood transmitted diseases could be avoided. This case highlights the importance of CBCT as planning tool for resection of the mandible, using piezoelectric knife as safe method to achieve bloodless surgery.

Key words:
mandibular neoplasms; hemangioma; diagnosis; cone-beam computed tomography; oral surgical procedures; bone transplantation.

Apstrakt

Uvod. Intraosealna vaskularna malformacija vilica može ugroziti život bolesnika zbog nekontrolisanog krvarenja do kojeg može doći nakon ekstrakcije zuba. Shodno biološkom ponašanju tih lezija, primena odgovarajućih dijagnostičkih i terapijskih metoda neophodna je kako bi se izbegle moguće komplikacije. Prikazana je *en bloc* resekcija intraosealne venske malformacije piezoelektričnim nožem nakon prethodno izvršene kompjuterizovane tomografije konusnim zrakom (CBCT). **Prikaz bolesnika.** Kod bolesnika starosti 55 godina resekcija intraosealne fokalne vaskularne malformacije donje vilice piezoelektričnim nožem urađena je nakon prethodne CBCT, a defekt je rekonstruisan slobodnim koštanim transplantatom sa ilijačne kosti. Hirurško lečenje i postoperativni tok protekli su bez komplikacija. U periodu praćenja od dve godine nije došlo do razvoja recidiva. **Zaključak.** Piezoelektričnim nožem može se postići precizna i bezbedna resekcija uz minimalan gubitak krvi, što je od posebnog značaja kada je u pitanju venska malformacija koštanog tkiva. Prednosti ove tehnike su: mali rizik od oštećenja mekotkivnih struktura, mogućnost precizne osteotomije i minimalan gubitak krvi. Korišćenjem piezoelektričnog noža može se izbeći transfuzija krvi kao i transmisija krvnoprenosivih bolesti. U ovom prikazu slučaja posebno je istaknut značaj planiranja resekcije donje vilice piezoelektričnim nožem pomoću CBCT. To je izuzetno bezbedan metod lečenja, kojim se gubitak krvi svodi na minimum.

Ključne reči:
mandibula, neoplazme; hemangiom; dijagnoza; kompjuterizovana tomografija konusnim zrakom; hirurgija, oralna, procedure; transplantacija kosti; lečenje, ishod.

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Introduction

Head and neck vascular anomalies are classified according to the International Society for the Study of Vascular Anomalies (ISSVA) into two groups: vascular tumors and vascular malformations [1]. In 1982 Mulliken and Glowacki [2] proposed the classification of vascular anomalies in order to standardize the nomenclature. Vascular malformations are present at birth and enlarge proportionately with the growth of the child and do not undergo spontaneous involution [2, 3]. Hemangiomas, considered as vascular tumors, have two main types: infantile hemangiomas (IH) and congenital hemangiomas. There are two types of congenital hemangiomas: rapid involuting congenital hemangioma (RICH) and non-involuting congenital hemangioma (NICH). Hemangiomas generally are characterised by spontaneous involution, except non-involuting congenital hemangioma (NICH). Applying the classification of the ISSVA and the classification of vascular tumors of bone of the World Health Organization (WHO), intraosseous cavernous hemangioma (ICH) corresponds to intraosseous focal venous malformation (IFVM) [4–6]. This revised and updated nomenclature is used in this article.

Intraosseous vascular lesions of the mandible are usually asymptomatic, but they can be present as uncomfortable slow growing lesions with hard consistence, spontaneous hemorrhage, pulsatile sensation or tooth mobility [7, 8]. Clinical signs may include swelling of the soft tissues, pain of varying intensity, change in the color of the oral mucosa, toothache, unexplained bleeding of gums and enlargement of the cortical plate that makes the jaw asymmetrical [7, 9]. Among distortion, destruction, hypertrophy, hypoplasia and density change, primary intraosseous vascular lesions were also reported as possible alterations of the skeleton [10]. Radiographically they range from an unilocular rounded lesion, "honeycombed", "sunburst" or radiopaque appearance [8]. Differential diagnoses include odontogenic tumors, ameloblastomas, cystic lesions and fibrous dysplasia [7, 9, 11, 12].

Cone beam computed tomography (CBCT) has been commercially available as diagnostic tool since 2001. It has been used firstly in oral surgery and maxillofacial surgery giving the possibility of adequate visualisation of bony structure and pathology [13–16].

Piezosurgery create bloodless surgical site that makes visibility in the working area much clearer than with conventional bone cutting instruments. Unlike conventional burs and micro saws, piezosurgery inserts do not become hot, which again reduces the risk of postoperative necrosis. Nowadays, piezosurgery is one of the most advanced technologies in dental surgery, but also could become very important in head and neck surgery.

By minimising blood loss during surgery, piezosurgery could be very helpful tool in order to avoid blood transfusion.

We presented an urgent *en bloc* resection of an intraosseous venous malformation by a piezoelectric knife based on CBCT planning.

Case report

A 55-year-old asymptomatic male was referred to the Maxillofacial Surgery Department in Belgrade by his dentist to investigate a radiolucent lesion of the left mandibular body that he accidentally noticed using orthopantomography (OPG) prior tooth extraction (Figure 1). The patient complained of tooth loosening and lower denture instability due to a swelling underneath. There was no reported bleeding and the patient had no other systemic signs and symptoms and no comorbidities.

OPG showed an ill-defined multilocular lesion extending from parasymphiseal region to the angle of mandible (Figure 1).

As additional procedure and in order to minimise patient's radiation, CBCT (Planmeca unit promax 3DS) of the mandible was performed. It showed ill-defined multilocular osteolytic expansive lesion which was associated with mandibular canal. Both of the lingual and buccal mandibular cortical bones revealed signs of invasion (Figure 2).

After administrating of block and terminal (plexus) local anaesthesia (1 : 80,000 lidocaine with adrenaline) and raising of mucoperiosteal flap, non-pulsatile, dark blue soft tissue masses of mandibular body was presented. Biopsy was carried out using No 11 surgical blade. It was followed by excessive intraoperative bleeding. Hemostasis was temporarily achieved with iodoform gauze packing, hemostatic mucogingival sutures and external compressive bandage. Frozen section showed IFVM.

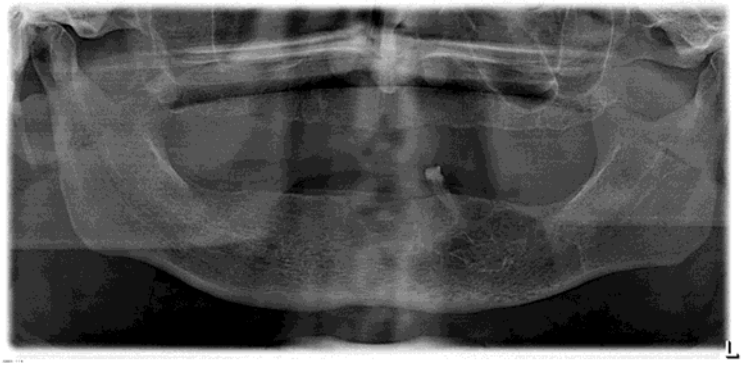

Fig. 1 – Orthopantomographic view showing discrete multilocular radiolucency in the left mandibular body.

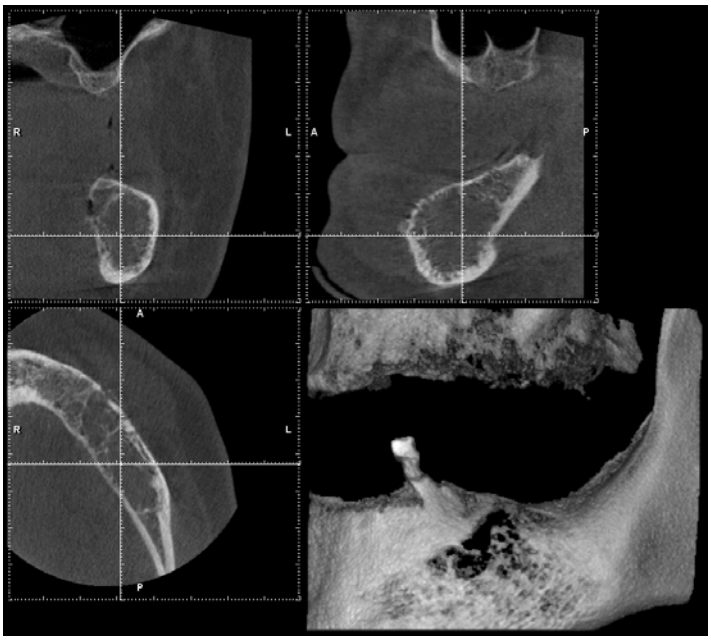

Fig. 2 – Core beam computed tomography (CBCT) of the mandible (Planmeca Unit Promax Cone Beam CT 3DS).

After obtaining the histological report, immediate treatment plan was made, using CBCT's prediction (On Demand Software Cybermed Seoul Korea). The patient was prepared and under hypotensive general anesthesia *en bloc* surgical resection of the mandibular body through submandibular approach was performed (Figure 3).

Piezosurgery was used to make precise surgical resection and to prevent excessive surgical bleeding. After achieving radical excision of the IFVM, left iliac bone graft was harvested and precisely remodelled by piezosurgery (Figures 4, 5). Figure 6 shows finding ofter free bone graft reconstruction of the mandible. Postoperative course was uneventful. Final histopathological report confirmed IFVM (Figure 7).

The 2-year follow-up revealed no signs of relapse. The patient was in the process of pre-implant planning.

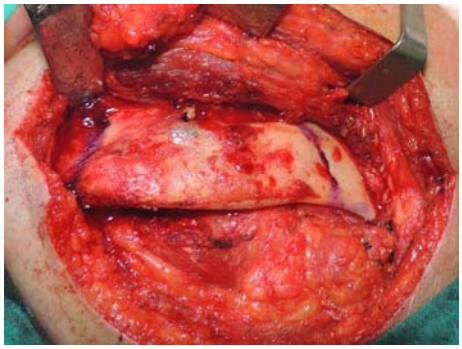

Fig. 3 – Submandibular approach to the mandibular body.

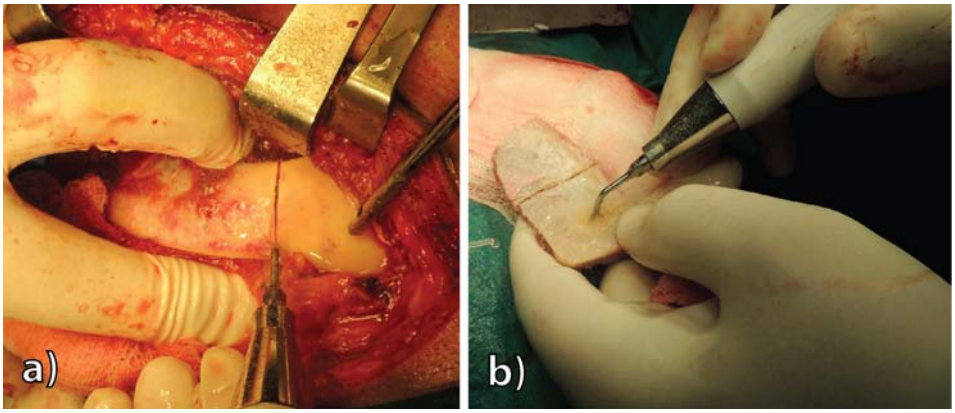

Fig. 4 – a) Use of piezoelectric knife in resection of intraosseous venous malformation (IVFM); b) Conturing iliac bone graft by use of piezoelectric knife.

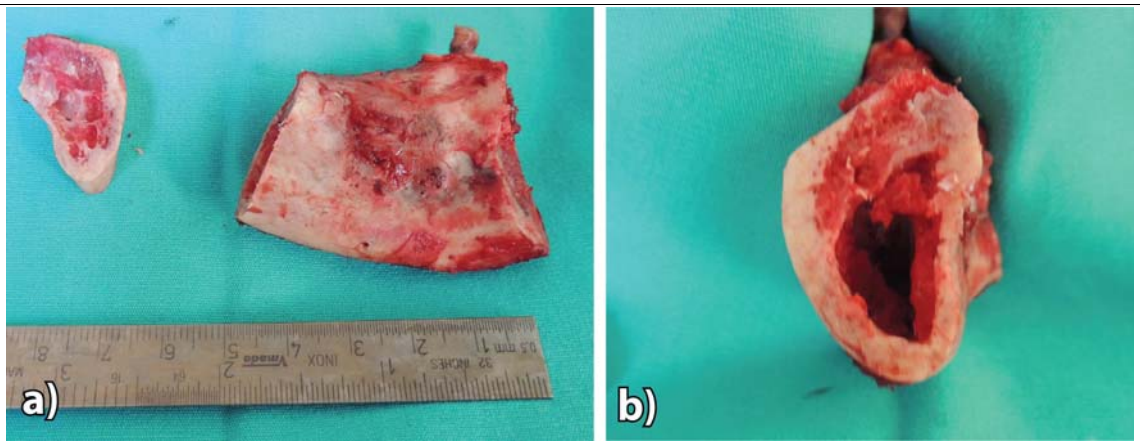

Fig. 5 – a) Surgical specimen; b) Cross section.

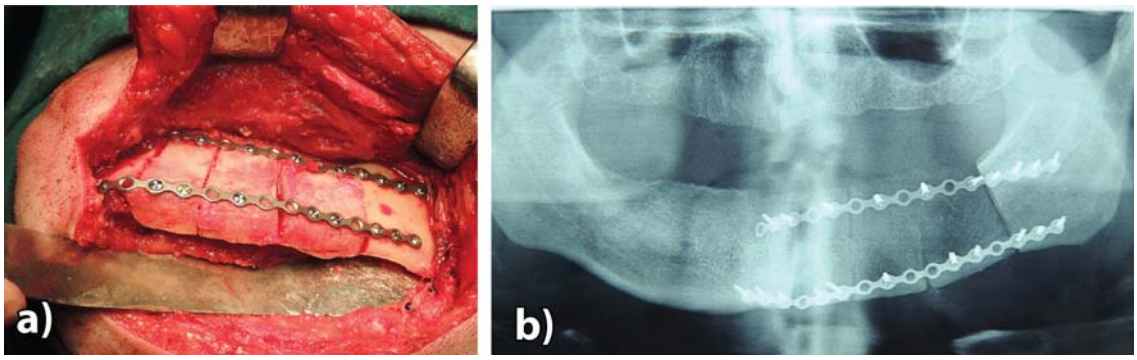

Fig. 6 – a) Iliac bone graft "in situ"; b) Postoperative orthopantomogram (OPG) X-ray.

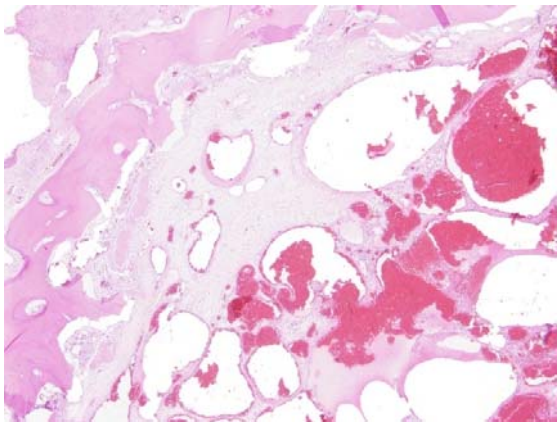

Fig. 7 – Intraosseous focal venous malformation (IFVM), which is composed of anastomosing, ectasic, engorged, thin-walled blood vessels (histologically-capillary hemangioma) (hematoxylin eosin, × 100).

Discussion

Historically "intrabony hemangioma" was presented as peripheral or central intraosseous lesion. It is classified as benign vascular tumor [17, 18] and accounts less than 1% of intraosseous "tumors" [3]. The highest incidence of occurring is in 20–50 years of age and it is almost twice more frequent in female population [8]. These lesions are commonly located in spine and skull bones, rarely in facial bones (zygomatic, orbital, mandible) [7, 12, 18–23].

The term "hemangioma" used in the literature to describe intraosseous vascular anomalies is a source of confusion [24]. Kaban et al. [11] reported that hemangiomas are not localized in the bone and postulated that "intrabony" vascular lesions are mostly venous malformations. Applying the classification of the ISSVA for an effective communication with medical doctors who are dealing with intraosseous vascular malformations, the term of IFVM was used in this article [25].

Usually detailed medical history and examination of a patient are sufficient to establish the clinical diagnosis of vascular anomalies of soft tissue [26]. However, IFVM of the jaws is rare lesion hidden in bone that could be difficult to diagnose. Because of the presence of multilocular radiolucenc, it can be often misdiagnosed as dentigerous cyst, ameloblastoma, central giant cell granuloma, myxoma and metastatic tumor, as well. The following signs and symptoms can be associated with the growth of IFVM: discomfort, pulsatile sensation, numbness, loosening the teeth, bluish discoloration of the

overlying mucosa [7, 8]. The most frequent localization of the IFVM in the mandible is in the premolar-molar region [27].

In this case the patient presented swelling in the mandibular area with no history of bleeding or altered neurological function. Radiologically there was ill-defined radiolucency in the premolar and molar region affecting both of the lingual and buccal mandibular cortical bones. Vascular malformation has to be taken in consideration in the differential diagnosis of any multi- or unilocular radiolucency of the jaws, particularly if there is the presence of "spoke like" and "sun ray" [28]. Clinically and radiologically IFVM could present as many other pathological entities in lower jaw.

Until now there is no single reliable non-invasive imaging technique that is adequate enough to diagnose venous malformation of the bone tissue [29].

Computed tomography (CT) and magnetic resonance (MR) angiography could be used in the diagnosis of vascular malformations (flow and feeding vessels characteristics) [12, 18], but it requires time for patient preparation and usually is not available technique for urgent situations. CBCT technology is clinically introduced in 1998 [29–31], and due to a high resolution imaging possibilities without using contrast became alternative to multislice CT and event better tool than MR [14, 18]. New generation of CBCT presented not only usability in establishing the diagnosis of IFVM but also enable clear visualization of the cortical involvement and relation with surrounding structures [18]. In this case CBCT has been used to plann *en bloc* segmental resection of the mandibular lesion and enable immediate reconstruction of the defect with free bone graft. CBCT was of great importance in giving to the surgeon accurate information about the extension of the lesion and the prediction of the margins of resection's.

With the rapid development of head and neck surgical techniques over the last few decades [32, 33] this surgical technique based on piezoelectric phenomenon seems to have a lot of applications [34, 35], but until now piezoelectric resection of the IFVM of the mandible is not yet presented in the literature, except creating window for embolisation in two cases of arterio-venous malformation [36].

This article reports an urgent treatment of IFVM in the mandibular body because of acute and excessive bleeding after biopsy attempt and usage of piezosurgery enabled surgeon to create almost bloodless surgical field. It also carries minimal risk of bone necrosis due to a constant cooling in comparison to conventional surgical instruments such as burs and micro saws. Beside wide usage in dentistry piezosurgery also became a power tool in head and neck surgery. Using this ultrasonic knife the possibility of damaging blood vessels and nerves is decreased because a piezoknife only cuts hard tissue (bony structures). According to the pathology treated in this case it was very important to provide safe and bloodless procedure as much as possible.

Therapeutic alternatives in the treatment of intraosseous venous malformation include curettage and sclerotherapy [2, 26].

Conclusion

Intraosseal venous focal malformation in orofacial region should be always considered in the differential diagnosis of multilocular intraosseal lesions in oral and maxillofacial region!

The use of CBCT in the diagnosis and radiographic planning of resection of intraosseal venous focal malformation of the mandible is new method and gives more precise information not only about nature of vascular anomaly, but also of extension inside bone structure. This enables surgeon to make precise plan of resection and avoid unnecessary bleeding in potentially life threatening situation.

The advantages of piezosurgery in the treatment of intraosseal vascular malformation of the mandible are: lower risk of damaging soft tissue structures, precise osteotomy and bloodless surgery. Moreover, using piezosurgery, blood transfusion and blood transmitted diseases could be avoided.

Acknowledgements

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R E F E R E N C E S

1. *Enjolras O, Wassef M, Chapot RO.* Color Atlas of Vascular Tumors and Vascular Malformations, 1st ed. West Nyack (NY): Cambridge University Press; 2009.
2. *Mulliken JB, Glowacki J.* Hemangiomas and vascular malformations in infants and children: A classification based on endothelial characteristics. Plast Reconstr Surg 1982; 69(3): 412−22.
3. *Adler CP, Wold L.* Heamangioma and related lesions. In: *Fletcher CD, Unni KK, Mertens F,* editors. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press; 2002. p. 320−1.
4. *Bruder E, Perez-Atayde AR, Jundt G, Alomari AI, Rischewski J, Fishman SJ,* et al. Vascular lesions of bone in children, adolescents, and young adults. A clinicopathologic reappraisal and application of the ISSVA classification. Virchows Arch 2009; 454(2): 161−79.
5. *Stošić S, Anđelić G, Tomić LJ.* Contemporary concept in classification of vascular anomalies of head and neck. MD-Medical Data 2011; 3(4): 383−6.
6. *Stošić S.* Vascular anomalies of head and neck. Belgrade: Odbrana; 2012. (Serbian)
7. *Cheng N, Lai D, Hsie M, Liao S, Chen YT.* Intraosseous hemangiomas of the facial bone. Plast Reconstr Surg 2006; 117(7): 2366−72.
8. *Gomez Oliveira G, García-Rozado A, Luaces Rey R.* Intraosseous mandibular hemangioma. A case report and review of the literature. Med Oral Patol Oral Cir Bucal 2008; 13(8): E496−8.
9. *Sun Z, Yang L, Yi C, Zhao H, Han D, Yang T,* et al. Possibilities and potential roles of estrogen in the pathogenesis of proliferation hemangiomas formation. Med Hypotheses 2008; 71(2): 286−92.

10. *Boyd JB, Mulliken JB, Kaban LB, Upton J, Murray JE.* Skeletal changes associated with vascular malformations. Plast Reconstr Surg 1984; 74(6): 789−97.
11. *Kaban LB, Mulliken JB.* Vascular anomalies of the maxillofacial region. J Oral Mmaxillofac Surg 1986; 44(3): 203−13.
12. *Eliot CA, Castle JT.* Intraosseous hemangioma of the anterior mandible. Head Neck Pathol 2010; 4(2): 123−5.
13. *Nakagawa Y, Kobayashi K, Ishii H, Mishima A, Ishii H, Asada K,* et al. Preoperative application of limited cone beam computerized tomography as an assessment tool before minor oral surgery. Int J Oral Maxillofac Surg 2002; 31(3): 322−6.
14. *Danforth RA.* Cone beam volume tomography: A new digital imaging option for dentistry. J Calif Dent Assoc 2003; 31(11): 814−5.
15. *Hatcher DC, Dial C, Mayorga C.* Cone beam CT for pre-surgical assessment of implant sites. J Calif Dent Assoc 2003; 31(11): 825−33.
16. *Brown J, Chatterjee R, Lowe D, Lewis-Jones H, Rogers S, Vaughan D.* A new guide to mandibular resection for oral squamous cell carcinoma based on the Cawood and Howell classification of the mandible. Int J Oral Maxillofac Surg 2005; 34(8): 834−9.
17. *Prein J, Remagen W, Spiessl B, Uehlinger E.* Atlas of tumors of the facial skeleton: Odontogenic and nonodontogenic tumors. Berlin-Heidelberg: Springer; 1986.
18. *Kalsi HK, Scannel J.* Unusual presentation of an intraosseous haemangioma of the maxilla and displaced canine. Oral Surgery 2013; 6(2): 80−2.
19. *Williams HJ, Wake MJ, John PR.* Intraosseous haemangioma of the mandible: A case report. Pediatr Radiol 2002; 32(8): 605−8.
20. *Valentini V, Nicolai G, Lorè B, Aboh IV.* Intraosseous Hemangiomas. J Craniofac Surg 2008; 19(6): 1459−64.
21. *Hansen T, Kunkel M, Katenkamp D, Eletr S, Wagner W.* Hemangioma of the mandible: Case report with special emphasis on bone degradation. Oral Maxillofac Surg 2009; 13(4): 239−42.
22. *Wu J, Du J, Lu P, Chen C.* Interdisciplinary management of mandibular intraosseous haemagioma. Br J Oral Maxillofac Surg 2011; 49(7): e55−7.
23. *Alves S, Junqueira JL, Oliveira EM, Pieri SS, Magalhães MH, Dos Santos Pinto D,* et al. Condylar hemangioma: Report of a case and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102(5): e23−7.
24. *Greene AK, Rogers GF, Mulliken JB.* Intraosseous "hemangiomas" are malformations and not tumors. Plast Reconstr Surg 2007; 119(6): 1949−50.
25. *Lowe LH, Marchant TC, Rivard DC, Scherbel AJ.* Vascular malformations: Classification and terminology the radiologist needs to know. Semin Roentgenol 2012; 47(2): 106−17.
26. *Ernemann U, Kramer U, Miller S, Bisdas S, Rebmann H, Breuninger H,* et al. Current concepts in the classification, diagnosis and treatment of vascular anomalies. Eur J Radiol 2010; 75(1): 2−11.
27. *Drage NA, Whaites EJ, Hussain K.* Haemangioma of the body of the mandible: A case report. Br J Oral Maxillofac Surg 2003; 41(2): 112−4.
28. *Zlotogorski A, Buchner A, Kaffe I, Schwartz-Arad D.* Radiological features of central haemangioma of the jaws. Dentomaxillofac Radiol 2005; 34(5): 292−6.
29. *Dreiseidler T, Alarabia N, Ritter L, Rothamel D, Scheer M, Zöller JE,* et al. A comparison of multislice computerized tomography, cone-beam computerized tomo-graphy and single photon emission computerized tomography for the assessment of bone invasion by oral malignancies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(3): 367−73.
30. *Mozzo P, Procacci C, Tacconi A, Martini PT, Andreis IA.* A new volumetric CT machine for dental imaging based on the cone-beam technique: Preliminary results. Eur Radiol 1998; 8(9): 1558−64.
31. *Hendrikx AF, Maal T, Dieleman F, Van Cann EM, Merkx MW.* Cone-beam CT in the assessment of mandibular invasion by oral squamous cell carcinoma: Results of the preliminary study. Int J Oral Maxillofac Surg 2010; 39(5): 436−9.
32. *Moore SL, Chun JK, Mitre SA, Som PM.* Intraosseous hemangioma of the zygoma: CT and MR findings. AJNR Am J Neuroradiol 2001; 22(7): 1383−5.
33. *Perugini M, Renzi G, Gasparini G, Cerulli G, Becelli R.* Intraosseous hemangioma of the maxillofacial district: Clinical analysis and surgical treatment in 10 consecutive patients. J Craniofac Surg 2004; 15(6): 980−5.
34. *Landes CA, Stübinger S, Laudemann K, Rieger J, Sader E.* Bone harvesting at the anterior iliac crest using piezoosteotomy versus conventional open harvesting: A pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105(3): e19−28.
35. *Degerliyurt K, Akar V, Denizci S, Yucel E.* Bone lid technique with piezosur-gery to preserve inferior alveolar nerve. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(6): e1−5.
36. *Lemound J, Brachvogel P, Götz F, Rücker M, Gellrich N, Eckardt A.* Treatment of mandibular high-flow vascular malformations: Report of 2 cases. J Oral Maxillofac Surg 2011; 69(7): 1956−66.

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CASE REPORT

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Hereditary hemorrhagic telangiectasia associated with inherited thrombophilia

Nasledna hemoragijska teleangiektazija udružena sa naslednom trombofilijom

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Abstract

Introduction. Hereditary hemorrhagic telangiectasia and inherited thrombophilia are genetic disorders with quite opposite clinical manifestation. The main characteristic for hereditary hemorrhagic telangiectasia is recurrent bleeding, while the main characteristic for hereditary thrombophilia is thrombosis. The association between hereditary hemorrhagic telangiectasia and inherited thrombophilia in the same patient is rare. **Case report.** We presented a 32-year-old female with recurrent gastrointestinal hemorrhage and epistaxes, during a 9-year period. Hereditary hemorrhagic telangiectasia was established according to "Curaçao" criteria. Three of four criteria have been present: spontaneous recurrent epistaxis, multiple telangiectasias (nose) and visceral lesions (gastric angiodysplasias, jejunal telangiectasias, arterio-venous jejunal fistula). Pulmonary thromboembolism was the first manifestation of thrombophilia; the diagnosis was confirmed by genetic testing. Therapy of hemorrhage with tranexamic acid (anti-fibrinolytic agent; its use increases risk of thrombosis) was unsuccessful. Remission was achieved by thalidomide. The initial therapy for pulmonary thromboembolism included aspirin (that have an increased risk of bleeding), but aspirin had to be discontinued because of massive hematemesis. Unfortunately, a year later, anticoagulant therapy combined with the proton pump inhibitors, were introduced, because of a new thrombosis. One month after, the patient was still on this therapy, without new episodes of bleeding and thromboembolic events. **Conclusion.** Hereditary hemorrhagic telangiectasia and inherited thrombophilia could be unrecognized for years, partly due to the lower degree of clinical suspicion. Early diagnosis and the appropriate choice of therapy are essential for reducing serious consequences and to improve quality of life.

Key words:
telangiectasia, hereditary hemorrhagic; thrombophilia; comorbidity; diagnosis; treatment outcome.

Apstrakt

Uvod. Nasledna hemoragijska teleangiektazija i nasledna trombofilija su genetski poremećaji sa potpuno suprotnim kliničkim manifestacijama. Glavna karakteristika nasledne hemoragijske teleangiektazije su ponavljana krvarenja, a glavna karakteristika nasledne trombofilije je tromboza. Udruženost oba poremećaja kod istog bolesnika retko se sreće. **Prikaz bolesnika.** Prikazali smo bolesnicu staru 32 godine, koja je tokom devet godina imala ponavljana gastrointestinalna krvarenja i epistakse. Dijagnoza nasledne hemoragijske teleangiektazije postavljena je prema „Curaçao" kriterijumima. Kod bolesnice su postojala tri, od četiri kriterijuma: spontane, ponavljane epistakse, multiple teleangiektazije (u nosu) i visceralne lezije (angiodisplastične promene u želucu, teleangiektazije i arterio-venska fistula u jejunumu). Prva manifestacija trombofilije bila je plućna tromboembolija, a dijagnoza je potvrđena genetskim testiranjem. Jednomesečna terapija krvarenja traneksamičnom kiselinom (antifibrinolitički agens čije korišćenje povećava rizik od tromboze) bila je neuspešna, pa je remisija postignuta talidomidom. Plućna tromboembolija inicijalno je lečena aspirinom (koji je lek sa povišenim rizikom od krvarenja), ali lečenje je prekinuto zbog masivne hematemeze. Godinu dana kasnije, zbog pojave novih tromboza, započeta je antikoagulantna terapija u kombinaciji sa blokatorima protonske pumpe. Tokom mesec dana terapije nije bilo novih epizoda krvarenja, niti tromboze. **Zaključak.** Nasledna hemoragijska teleangiektazija i nasledna trombofilija mogu biti neprepoznate godinama, delom i zbog toga što se na ove bolesti retko misli. Rana dijagnoza i izbor adekvatne terapije su od ključnog značaja za smanjivanje broja ozbiljnih posledica i poboljšanje kvaliteta života.

Ključne reči:
teleangiektazija, nasledna, hemoragijska; trombofilija; komorbiditet; dijagnoza; lečenje, ishod.

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Introduction

Hereditary hemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber disease is an autosomal dominant disorder of angiogenesis, presented by mucocutaneous and visceral telangiectasias and arteriovenous malformations in the lungs, liver, gastrointestinal tract, spinal cord, and the brain. Visceral arteriovenous malformations may cause serious, and sometimes lifethreatening complications, such as brain abscesses, strokes and hemorrhages [1–3]. The prevalence for HHT is of 1.5–2.0 /10,000 persons [4]. Clinical diagnosis is based on the "Curaçao" criteria [5], while genetic testing is indicated in asymptomatic patients with positive family history and in the patients with clinical suspected disease [6, 7].

Inherited thrombophilia is a genetic disorder caused by the lack of natural inhibitors of coagulation (antithrombin, protein C, protein S), or by gene mutations for factor V Leiden, prothrombin G20210A and methylene tetrahydrofolate reductase C667T [8, 9]. It represents a hypercoagulable state, leading to venous and arterial thrombosis. The most frequent clinical manifestations are deep vein thrombosis and pulmonary embolism [10, 11].

We presented a patient, suffered from HHT associated with inherited thrombophilia. In the current literature, we found only two similar cases so far [12, 13].

Case report

A 32-year-old female was admitted to our Clinic for the first time in January 2009, because of recurrent gastrointestinal hemorrhage (melena and hematemesis) and the consequent hypochromic anemia. Her previous medical history included purulent meningitis in 1996 and repeated episodes of epistaxis, bloody stools, hematemesis, metrorrhagia and hematuria, and Henoch Schonlein purpura between 2001 and 2006. Gastroenterological examination performed in 2004 in other institution revealed angiodysplasia in gastric mucosa without the signs of active bleeding. In July 2005, after cessation of pregnancy due to sepsis**,** the patient had a grand-mall seizure, but computed tomography (CT) brain scan was normal. On admission, the patient was pale, with normal coagulation status, but with low levels of hemoglobin [61 g/L – the normal range (NR) is 120–160 g/L], mean corpuscular volume – MCV [67 fL, NR 80–100 fL], serum iron (5 μmol/L, NR 9–30.4 μmol/L), and ferritin (31 μg/L, NR 50–200 μg/L). During the one month hospitalization, the patient had seven episodes of melena, hematemesis and epistaxis, resulted in severe hypochromic anemia (hemoglobin 60 g/L), which required blood transfusions. Frequently, gastrointestinal hemorrhage was followed by the grand-mall seizure. Gastric angiodysplasia without active bleeding was found by repeated endoscopy, and it was treated by argon plasma coagulation. Thoracic and brain CT, as well as abdominal ultrasound, gynecological and otorhinolaryngological examination were all normal. The patient was discharged in stable condition and recommended therapy included proton pump inhibitors and iron preparations.

The next hospitalization followed a week after discharge, due to abundant hematemesis, which was repeated several times in the hospital. Abdominal CT angiography revealed arteriovenous fistula between the I and II jejunal arteries and the I and II jejunal vein, in the jejunal wall distal to the ligament Treitz (Figure 1). The patient underwent jejunal resection in length of 70 cm and jejunojejunal anastomosis. Upon discharge, the patient continued to take proton pump inhibitors and iron preparations. In the following 16 months the patient had two episodes of appearance of blood in the stool lasting for 7 days each.

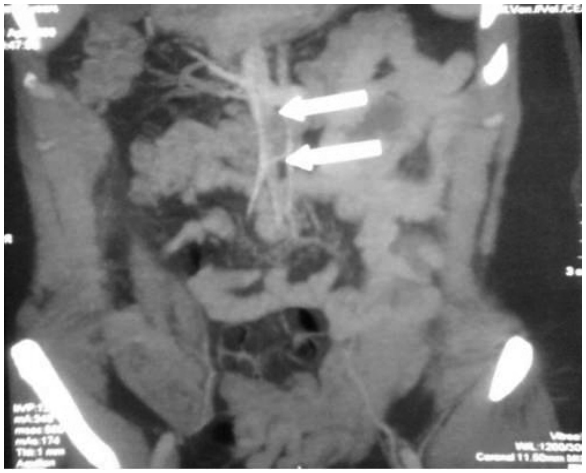

Fig. 1 – Abdominal computed tomography (CT) angiography: arteriovenous fistula between the I and II jejunal arteries and the I and II jejunal vein.

In September 2010 the patient was readmitted to our Clinic because of hematemesis and severe hypochromic anemia (hemoglobin 65 g/L). No sign of bleeding was found endoscopically, but selective angiography of visceral abdominal blood vessels revealed the presence of multiple telangiectatic lesions in jejunal mucose (Figure 2). Pulmonary and brain arteriovenous malformations were excluded by thoracic CT, cerebral magnetic resonance imaging (MRI) and cerebral angiography. The patient was considered to have HHT, and was commenced on tranexamic acid at a daily dose of 3 g. Bleeding tendency was not decreased and the therapy was discontinued after one month. After that, thalidomide therapy was initiated (100 mg twice daily), which stopped bleeding. The patient continued with the same therapy after discharge, but unfortunately not regularly.

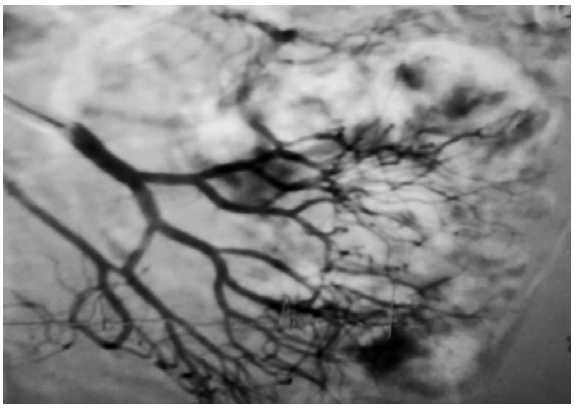

Fig. 2 – Selective angiography of visceral abdominal blood vessels: multiple telangiectatic lesions in jejunal mucosa.

In November 2011 the patient was hospitalized again because of hematemesis, anemia (hemoglobin 60 g/L), and diminished breath sounds over the right lung base. CT angiography of the chest diagnosed pulmonary thromboembolism (Figure 3). DNA analysis confirmed the dignosis of hereditary thrombophylia. The patient was found to be a homozygous carrier for methylene tetrahydrofolate reductase C667T gene mutation. In spite of bleeding tendency, we started with antiplatelet therapy after discharge with aspirin (100 mg twice weekly) and thalidomide 100 mg daily.

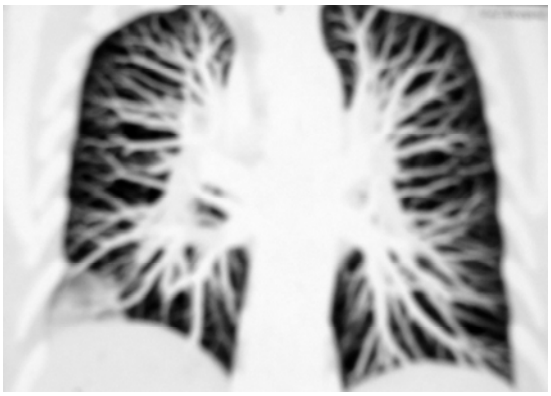

Fig. 3 – Computed tomography (CT) angiography of the chest: pulmonary thromboembolism.

In the next twenty months there were no episodes of bleeding. However, the patient stopped taking thalidomide therapy, which resulted in gastrointestinal hemorrhage and epistaxis, 1.5 months after the cessation of the therapy. This was the reason for new hospitalization in July 2013. During that hospitalization, the patient had hematemesis and melena every two or three days. External carotid angiography revealed numerous telangiectasia in the posterior part of the middle concha and in the nasopharynx (Figure 4).

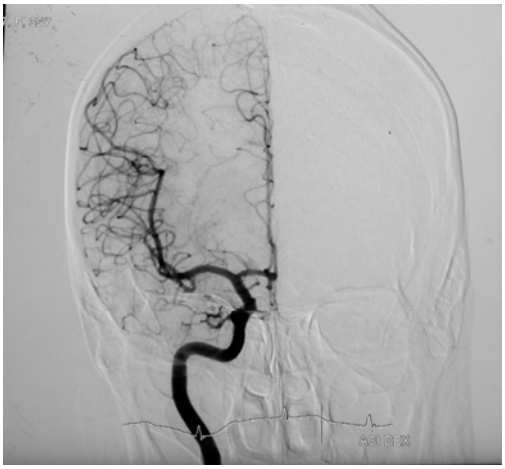

Fig. 4 – External carotid angiography: numerous telangiectasia in the posterior part of the middle concha and in the nasopharynx.

A low hemoglobin level (60 g/L) was the only biochemical disturbances. Clinical course was complicated with biliary colic and cholecystectomy was necessary. The initial therapy included aspirin and thalidomide, but aspirin had to be discontinued after one month because of massive hematemesis. After discharge, the patient had an episode of hematemesis (2014) with hemoglobin level of 82 g/L and an episode of epistaxis (March 2015). The patient was admitted to the hospital in March 2015 because of neck pain. Color duplex scan of the neck blood vessels detected thrombosis of external jugular veins bilaterally. Lung scanning showed small perfusion scan defects, possibly due to pulmonary thromboembolism. It was decided to start anticoagulant therapy (debigatran etexilate tbl, 110 mg/12 h), combined with the proton pump inhibitors. One month after, in April 2015, the patient was still on this therapy, without new episodes of bleeding and thromboembolic events.

Discussion

Hereditary thrombophilia and HHT are disorders with quite different clinical manifestation. The main characteristic for HHT is recurrent bleeding, most frequently from the nose and gastrointestinal tract. Epistaxis, from mild to severe, is usually the first manifestation in preschool children, although it may appear later, after thirty years of life. It is present in almost 90% of patients [1, 6, 14]. Gastrointestinal hemorrhage with chronic anemia is present in 15–30% of patients with HHT, and frequency increases with age. The first appearance is usually between forty and fifty years of life. The causes of bleeding are mucosal telangiectasias, angiodysplasias, or arteriovenous malformations [15–18].

In the presented patient, epistaxis and gastrointestinal bleeding started in her 24-year. Unfortunately, the disease had been unrecognized for almost 9 years. The diagnosis was established according to the "Curaçao" criteria. Three of four criteria were present: spontaneous recurrent epistaxis, multiple telangiectasias (nose), and visceral lesions: gastric angiodysplasias, jejunal telangiectasias, arterio-venous jejunal fistula. Some other authors have reported on a long diagnostic delay in patients with HHT [19, 20]. Interestingly, in the study of Pierucci et al. [19] diagnostic delay was 25.7 years.

Some studies demonstrated that patients with HHT are under "increased risk for both events: bleeding and clotting". Increased levels of coagulant factors V, VIII, and von Willebrand factor in HHT, result in enhance thrombotic risk in those patients [21, 22].

We did not find such abnormalities in the presented patient.

The main characteristic for thrombophilia is thrombosis, which may occur in different sites [23, 24]. Some authors pointed on increased risk for deep venous thrombosis and vascular gestational abnormalities in the patients with inherited thrombophilia [8, 25, 26]. In light of that, pregnancy loss in the presented patient in her 28, might be a complication due to thrombophilic gene mutation. However, the diagnosis was established 6 years later by finding the signs for pulmonary thromboembolism on chest CT angiography and by genetic testing.

Although patients with HHT may have increased risk for thrombosis, reports on the association between HHT and inherited thrombophilia in the same patient have been rare [12, 13]. There is a dilemma regarding the treatment of these patients, because of the opposite clinical manifestations of both diseases. Tranexamic acid, an antifibrinolytic agent, reduces epistaxis,

and to a lesser extent gastrointestinal bleeding, but caution is needed because of possible thrombotic events [14, 17, 27, 28]. Wechalekar and Parapia [13] successfully treated their patient, who had HHT associated with inherited thrombophilia, with tranexamic acid for 3 years. In the presented patient one month therapy with tranexamic acid was unsuccessful. Remission was achieved by thalidomide. In a few recent studies, the use of thalidomide, as inhibitor of angioneogenesis, has been recommended for the treatment of gastrointestinal bleeding in HTT [2, 17]. Another problem in the presented patient was the therapy of pulmonary thromboembolism. Acetylsalicylic acid (aspirin) was stopped after 20 months because of gastrointestinal hemorrhage. A year later, anticoagulant therapy combined with the proton pump inhibitors, was introduced, because of a new thrombosis. We have no other experience with such patients, but the results of the other authors suggest that the "long time anticoagulant therapy in patients with HHT is possible" [13, 29]. Wechalekar and Parapia [13] reported the second patient with HHT and predominat thrombosis who was on oral anticoagulant for 5 years, without bleeding or new thrombosis [13]. Bianca et al. [12] suggest that anticoagulant therapy is indicated in pregnant women with HHT and inherited thrombophilia, but obligatory hematological and clinical monitoring are required.

Conclusion

The association between HHT and inherited thrombophilia is a rare disorder. It could be unrecognized for years, partly due to the lower degree of clinical suspicion. The early diagnosis and appropriate choice of therapy are essential to reduce serious consequences and to improve quality of life.

Acknowledgement

The authors wish to thank Dr Siniša Rusović, who performed angiographic examinations, and also to the colleagues from the Clinic for Gastroenterology and Hepatology, Clinic for Urgent Internal Medicine and Intensive Care Unit of the Military Medical Academy in Belgrade, who took part in the treatment of the presented patient.

REFERENCES

1. *Duffau P, Lazarro E, Viallard JF.* Hereditary hemorrhagic telangiectasia. Rev Med Interne 2014; 35(1): 21−7. (French)
2. *Kapur KN, Morin JK, Letarte M.* Endoglin: A critical mediator of cardiovascular health. Vasc Health Risk Manag 2013; 9:195−206.
3. *Soubrier F, Chung WK, Machado R, Grunig E, Aldred M, Geraci M,* et al. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 2013; 62(25 Suppl): D13−21.
4. *Grosse SD, Boulet SL, Grant AM, Hulihan MM, Faughnan ME.* The use of US health insurance data for surveillance of rare disorders: Hereditary hemorrhagic telangiectasia. Genet Med 2014; 16(1): 33−9.
5. *Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ,* et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 2000; 91(1): 66−7.
6. *Govani FS, Shovlin CL.* Hereditary haemorrhagic telangiectasia: A clinical and scientific review. Eur J Hum Gen 2009; 17(7): 860−71.
7. *Botella LM, Albiñana V, Ojeda-Fernandez, L Recio-Poveda L, Bernabèu C.* Research on potential biomarkers in hereditary hemorrhagic telangiectasia. Front Genet 2015; 6: 115.
8. *Rambaldi MP, Mecacci F, Guaschino S, Paidas MJ.* Inherited and acquired thrombophilias. Reprod Sci 2014; 21(2): 167−82.
9. *Schellong SM.* Importance of thrombophilia screening. Internist (Berl) 2014; 55(5): 529−30; 532−4, 536. (German)
10. *Pomero F, Ageno W, Serraino C, Borretta V, Gianni M, Fenoglio L,* et al. The role of inherited thrombophilia in patients with isolated pulmonary embolism: A systematic review and a meta-analysis of the literature. Thromb Res 2014; 134(1): 84−9.
11. *Martinelli I, De Stefano V, Mannucci PM.* Inherited risk factors for venous thromboembolism. Nat Rev Cardiol 2014; 11(3): 140−56.
12. *Bianca S, Cutuli N, Bianca M, Barrano B, Cataliotti A, Barone C,* et al. Hereditary haemorrhagic telangiectasia and genetic thrombophilia. Eur J Hum Genet 2010; 18(4): 405−6.
13. *Wechalekar A, Parapia L.* Hereditary haemorrhagic telangiectasia with protein S deficiency in a family: A case report. Eur J Haematol 2000; 64(1): 59−60.
14. *Zarrabeitia R, Albiñana V, Salcedo M, Señaris-Gonzalez B, Fernandez-Forcelledo J, Botella L.* A review on clinical management and pharmacological therapy on hereditary haemorrhagic telangiectasia (HHT). Curr Vasc Pharmacol 2010; 8(4): 473−81.
15. *Kjeldsen AD, Kjeldsen J.* Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia. Am J Gastroenterol 2000; 95(2): 415−8.
16. *Ha M, Kim YJ, Kwon KA, Hahm KB, Kim M, Kim DK,* et al. Gastric angiodysplasia in a hereditary hemorrhagic telangiectasia type 2 patient. World J Gastroenterol 2012; 18(15): 1840−4.
17. *Kamath N, Bhatia S, Singh H, Shetty A, Shetty S.* Hereditary hemorrhagic telangiectasia. N Am J Med Sci 2015; 7(3): 125−8.
18. *Amanzada A, Töppler GJ, Cameron S, Schwörer H, Ramadori G.* A case report of a patient with hereditary hemorrhagic telangiectasia treated successively with thalidomide and bevacizumab. Case Rep Oncol 2010; 3(3): 463−70.
19. *Pierucci P, Lenato GM, Suppressa P, Lastella P, Triggiani V, Valerio R,* et al. A long diagnostic delay in patients with hereditary haemorrhagic telangiectasia: A questionnaire-based retrospective study. Orphanet J Rare Dis 2012; 7: 33.
20. *Sabbà C.* A rare and misdiagnosed bleeding disorder: Hereditary hemorrhagic telangiectasia. J Thromb Haemost 2005; 3(10): 2201−10.
21. *Shovlin CL.* Hereditary haemorrhagic telangiectasia: Pathophysiology, diagnosis and treatment. Blood Rev 2010; 24(6): 203−19.
22. *Dittus C, Streeiff M, Ansell J.* Bleeding and clotting in hereditary hemorrhagic telangiectasia. World J Clin Cases 2015; 3(4): 330−7.
23. *Cruz-Amy M, Hunter-Mellado R.* Factor V Leiden thrombophilia: presentation of three patients and a literature review. Bol Asoc Med P R 2006; 98(3): 213−21.
24. *Martinelli I, Passamonti SM, Bucciarelli P.* Thrombophilic states. Handb Clin Neurol 2014; 120: 1061−71.
25. *Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A,* et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999; 340(1): 9−13.

26. *Bleker SM, Coppens M, Middeldorp S*. Sex, thrombosis and inherited thrombophilia. Blood Rev 2014; 28(3): 123−33.
27. *Zaffar N, Ravichakaravarthy T, Faughnan ME, Shebata N*. The use of anti-fibrinolytic agents in patients with HHT: A retrospective survey. Ann Hematol 2015; 94(1): 145−52.
28. *Gaillard S, Dupuis-Girod S, Boutitie F, Rivière S, Morinière S, Hatron PY,* et al. Tranexamic acid for epistaxis in hereditary hemorrhagic telangiectasia patients: a European cross-over controlled trial in a rare disease. J Thromb Haemost 2014; 12(9): 1494−502.
29. *Edwards CP, Shebata N, Faughnan ME*. Hereditary hemorrhagic telangiectasia patients can tolerate anticoagulation. Ann Hematol 2012; 91(12): 1959−68.

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CASE REPORT

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Hemophagocytic syndrome triggered by intense physical activity and viral infection in a young adult female with three heterozygous mutations in Munc-18-2

Hemofagocitni sindrom izazvan intenzivnom fizičkom aktivnošću i virusnom infekcijom kod mlade odrasle ženske osobe sa tri heterozigotne mutacije u Munc-18-2

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Abstract

Introduction. Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially life-threatening, hyperinflammatory syndrome caused by severe hypercytokinemia due to a highly stimulated, but ineffective immune response. **Case report**. We reported a 19-year-old woman presenting with fever, muscle and joint pain and sore throat. After diagnostic procedures we made the diagnosis of hemophagocytic lymphohistiocytosis (7 of 8 HLH-2004 diagnostic criteria) caused by Ebstein-Barr viral infection and trigerred by the intense physical activity. Genetic analysis showed three different sequence changes in Munc-18-2, two splice acceptor side mutations/changes affecting exon 10 (c.795–4 C > T) and exon 15 (c.1247–10 C > T) and a missense mutation c.1375 C > T; p.Arg 459 Trp. All mutations were in heterozygous state and their significance in pathogensis of HLH is not clear. After treatment with corticosteroids and cyclosporin A complete clinical remission was achieved. **Conclusion.** The presented case history suggests the possibility that mutations of undetermined clinical significance in a gene associated with primary HLH may underlie some cases of secondary HLH, probably by causing a partial, rather than total or subtotal, impairment of encoded protein function. Our case also suggests that strenuous physical activity (in apparent synergy with viral infection) can trigger HLH.

Key words:
lymphohistiocytosis, hemophagocytic; inflammation; immunologic factors; physical exertion; ebstein-barr virus infections; mutation; diagnosis, differential; drug therapy.

Apstrakt

Uvod. Hemofagocitna limfohistiocitoza (HLH) je redak, moguće i životno ugrožavajći, upalni sindrom izazvan povećanom citokinskom aktivnošću nastalom na terenu izuzetno stimulisanog, ali neefikasnog imunskog odgovora. **Prikaz bolesnika.** Prikazali smo 19-godišnju bolesnicu koja je hospitalizovana zbog povišene temperature, bolova u mišićima i zglobovima i bolova u grlu. Nakon kompletne dijagnostičke obrade utvrđeno je da se radi o hemofagocitnoj limfohistiocitozi (7 od 8 HLH-2004 dijagnostičkih kriterijuma) uzrokovanoj infekcijom Ebstein-Barr-ovim virusom i intenzivnom fizičkom aktivnošću. Genetska analiza pokazala je tri heterozigotne mutacije u genu Munc-8-2, dve *splice* mutacije u egzonu 10 (c.795–4 C > t) i egzonu 15 (c.1247–10, C > T) i jednu *missence* mutaciju c. 1375 C > T; p.Arg 459 Trp, čiji patogenetski značaj nije jasan. Posle lečenja sa kortikosteroidima i ciklosporinom A ostvarena je kompletna klinička remisija. **Zaključak**. Ovaj prikaz bolesnice ukazuje na to da otkrivene heterozigotne mutacije (čiji patogenetski značaj nije utvđen) u genu Munc-18-2, inače povezanom sa primarnom HLH, mogu biti u osnovi i nekih sekundarnih HLH, izazivanjem delimičnog oštećenja funkcije kodiranog proteina. Ovaj slučaj takođe sugeriše da naporna fizička aktivnost (u očiglednoj sinergiji sa virusnom infekcijom) može biti pokretač HLH.

Ključne reči:
limfohistiocitoza, hemofagocitna; zapaljenje; imunski faktori; napor, fizički; ebstein-bar virus, infekcije; mutacija; dijagnoza, diferencijalna; lečenje lekovima.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal condition of immune system dysregulation characterized by severe inflammation and uncontrolled activation of T-cells and macrophages [1–3]. When the immune system is activated in a healthy person, histiocytes, natural killer (NK) cells and cytotoxic T lymphocytes (CTL) are all activated, further stimulating each other by receptor interactions as well as by secretion of inflammatory cytokines and chemokines [2, 3]. This leads to killing of infected cells, removal of antigen, and subsequent termination of the immune response. However, in HLH, there is a defect of NK and CTL function. Activated T lymphocytes and macrophages infiltrate organs and tissues and impair their function, with the occurrence of chronic inflammation caused by hypercytokinemia [3].

Depending on the presence or absence of an underlying condition, HLH can be either primary (genetic) or secondary (acquired) [4]. Primary HLH may be familial (FHLH) – or associated with some primary immunodeficiency [4]. Familial HLH is associated with mutations of genes involved in secretory pathways of cytotoxic lymphocytes, either through inactivation of granule contents or impairment of fusion, transport or delivery of such granules through the plasma membrane [5, 6]. Acquired HLH occurs with infections, lymphoproliferative disorders, metabolic and autoimmune diseases [7].

We presented a young adult female with HLH triggered by intense physical activity and viral infection, characterized by abnormal tests of T and NK cell degranulation and three heterozygous mutations in MUNC-18-2 gene.

Case report

A 19-year-old woman presented with a five-day history of fever (39ºC), muscle and joint pain and sore throat. A day before the appearance of symptoms the patient underwent an intense physical effort in the gym. She had no history of previous disease that could be the trigger for HLH. Three days after the initiation of symptoms antibiotic therapy started in primary care. As the fever persisted and the patient's condition deteriorated, the patient was admitted to the Clinic for Infectious Diseases (April 2012). Her personal and familial medical histories were unremarkable. Physical examination showed fever, a hyperemic pharynx, and cervical lymphadenopathy (maximal diameter 15 mm). Her blood count showed leukopenia and thrombocytopenia (hemoglobin – Hgb 134 g/L, white blood cell – WBC 3.8 × 10^9/L, platelet count – PLT 94 × 10^9/L) with normal leukocyte differential (neutrophils – Neu 54%, lymphocytes – Ly 40%, monocytes – Mon 6%). Laboratory analyses showed accelerated sedimentation of erythrocytes (SE) (24 mm/1h), elevated C-reactive protein – CRP (27.2 mg/L), ferritin (578 μg/L), aspartate aminotransminase – AST (718 U/L), alanine aminotransaminase – ALT (369 U/L), lactate dehydrogenase – LDH (2,086 U/L), creatine-kinase (3,423 U/L), normal holesterol (3.41 mmol/L) and triglyceride level (1.14 mmol/L). Haptoglobin was immeasurable. Tests of hemostasis showed normal prothrombin time – PT, activated partial thromboplastin time – aPTT and fibrinogen level, and increased D-dimer (4.37 μg/mL). Serum soluble interleukin-2 receptor and interleukin-6 were increased (4.86 ng/mL and 21 pg/mL, respectively; reference range 0.2–2.0 ng/mL, and 1.9–6.5 pg/mL, respectively). Serological analyses for human immunodeficiency virus – HIV, hepatitis B virus antigen – HbsAg, hepatitis C virus – HCV, hepatitis A virus – HAV and *Mycoplasma pneumoniae* were negative. Influenza A (H1, H3), Influenza B and respiratory syncytial virus (RSV) infections were not detected by polymerase chain reaction (PCR) analysis. Tumor markers alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were normal. Epstein-Barr virus (EBV) serology showed positive IgM antibodies and gradual increase in the titer of IgG from < 1/40 in the first sample to 1/160 in the third sample. EBV was not detected by PCR analysis, performed at two occasions. Immunological analyses: antinuclear antibodies (ANA), human epithelial line type 2 (HEp2), extractable nuclear antigens (ENA), circulating immune complexes (CIC), complement 3 (C3), complement 4 (C4), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), anti-neutrophil cytoplasmatic antibodies (ANCA) and anti-liver kidney microsomal antibodies (anti-LKM) were negative. Chest radiography revealed normal finding. Computed tomography scan visualized bilateral axillary lymphadenopathy (15–20 mm) and hepatosplenomegaly (liver 178 mm, spleen 185 mm). Bone marrow aspirate examination showed discrete hemophagocytosis. Morphological examination of the bone marrow trephine biopsy showed reactive changes. Pathohistological findings of an extirpated axillar lymph node showed sinus histiocytosis. Immunophenotyping of peripheral blood lymphocytes showed increased amount of activated (HLA-DR+) CD4+ (41.05%) and CD8+ (87.41%) T cells. As hemophagocytic syndrome was suspected, functional analysis of NK cells and CTL was performed (Centre for Chronic Immunodeficiency, University of Freiburg, Germany). NK cell cytotoxicity assay showed normal findings. Flow cytometry showed normal expression of perforin in NK cells. Analysis of activation-induced degranulation performed by detection of surface expression of CD107 on NK cells showed low, but not absent degranulation of NK cells after stimulation with target cells on two occasions (8.69%; 7.0%). No recovery of NK cell degranulation after stimulation with IL-2 was detected (11.09%). This finding was compatible with familial HLH. Repeated analysis of NK cell degranulation (one month later) showed a better result than in the first assay, but it was still not normal (28.95%). Degranulation of CTL after activation with anti-CD3/CD28 antibodies was normal in the first analysis (16.27%) and reduced in the second one (7.71%). According to all these findings and revised diagnostic guidelines for HLH (Table 1) [8] the diagnosis of HLH was established. Genetic analysis (performed at University Medical Center Hamburg, Germany) did not show changes in MUNC 13-4 gene, but three different sequence changes in

Table 1
Revised diagnostic guidelines for hemophagocytic lymphohistiocytosis (HLH)

The diagnosis HLH can be established if one the 2 below is fulfilled:
The molecular diagnosis consistent with HLH or
Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
Fever
Splenomegaly
Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood):
Hemoglobin < 90 g/L (in infants < 4 weeks: hemoglobin < 100 g/L)
Platelets < 100 × 109/L
Neutrophils < 1.0 × 109/L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides ≥ 265 mg/100 mL
Fibrinogen ≤ 1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
No evidence of malignancy (low or absent NK cell activity)
New diagnostic criteria
Ferritin ≥ 500 mg/L
Soluble CD25 (i.e., soluble IL-2 receptor) > 2,400 U/mL

NK – Natural killer.

Munc-18-2 were detected: two splice acceptor site mutations/changes affecting exon 10 (c.795–4 C > T) and exon 15 (c.1247–10 C > T) and a missense mutation c.1375 C > T; p.Arg459Trp, each of them in heterozygous state. The patient's mother was the carrier of the exon 10 splice site mutation and her father the carrier of the remaining two mutations. A splice site prediction program revealed no changes in splicing. The missense mutation had a low conservation grade. The overall pathogenetic significance of the detected mutations was unclear. At first, the patient was treated with symptomatic therapy. However, the patient's condition steadily deteriorated and laboratory analyses showed further aggravation. Therefore, the treatment consisting of corticosteroids, intravenous immunoglobulins, albumins and intravenous acyclovir was initiated. Soon after the initiation of treatment, the patient's condition gradually improved. However, as any attempt to reduce the dose of corticosteroids resulted in a drop of hemoglobin level, leukocyte and thrombocyte values, cyclosporin A (6 mg/kg) was introduced and the patient was transferred to the Hematology Department. In a few weeks fever subsided, her general condition improved, and all laboratory findings became normal. On the day 78 of hospitalization, the patient was discharged in good clinical condition. She thereafter remained, up to the time of writing (two years later) in excellent health.

Discussion

Primary hemophagocytic lymphohistiocytosis appears during infancy in 70–80% of the patients, usually within the first two years of life [9, 10]. Thanks to a better understanding of the genetic basis of the disease and better diagnostics, HLH has been increasingly diagnosed in patients presenting beyond infancy. These atypical presentations have been reported in adolescents and even in adults [3, 6]. Time of onset of disease and severity of clinical presentation are a reflection of underlying genetic aberrations and the intensity of triggering factors[1, 2]. Rohr et al. [9] classified a group of HLH patients presenting with HLH before two years of age as "typical FHLH" and the patients older than two years of age and survival without hematopoietic stem cell transplantation (HSCT) until at least six years of age as "atypical HLH" [10].

The presented patient met seven of the eight HLH-2004 diagnostic criteria (fever, splenomegaly, pancytopenia, hypofibrinogenemia, hemophagocytosis in bone marrow, abnormal NK cell activity, elevated ferritin and solubile IL-2 receptor). As immunological analyses were negative the diagnosis of myositis and other rheumatological diseases were excluded. Functional tests of NK cells and cytotoxic T lymphocytes showed findings that were not discriminative between primary and secondary HLH. Genetic analysis in our patient showed three different sequence changes of Munc-18-2 (syntaxin binding protein 2 – STXBP2) in heterozygous state. Syntaxin binding protein 2 (Munc-18-2) is involved in the regulation of vesicle transport to the plasma membrane [6]. According to the literature, some mutations in STXBP2 may be associated with milder and often recurrent HLH episodes and prolonged survival even without HSCT, which is unusual in the patients with the "typical" HLH [6]. However, clinical significance and

patogenetic potential of three different heterozygous sequence changes of Munc-18-2 gene detected in the presented patient is, at present, not clear (zur Stadt, personal communication). It is possible that these sequence changes decrease the threshold for the development of HLH, but to a lesser extent than severe mutations that cause HLH during the early stages of life.

When the diagnosis of hemophagocytic syndrome was established in our patient, search for an underlying infection, genetic, rheumatological, or malignant disease was undertaken. The presented patient had elevated creatin kinase which is an unusual finding in hemophagocytic syndrome, unless in the context of underlying myositis. However, the presence of rheumatological disease or malignant disease was excluded. Consequently, the diagnosis of HLH triggered by EBV infection was made. However, in addition to genetic predisposition and triggerring factors, additional factors that contribute to the development of HLH may exist. Such a factor in the presented patient was intense physical activity. Strenuous exercise induces negative changes in the cellular compartment of the immune system and suppression of NK cell cytotoxic activity [11]. Furthermore, strenuous exercise induces increased circulating levels of several cytokines [10]. Bruunsgaard et al. [12] show that post-exercise cytokine production is related to skeletal muscle damage. They found an association between increased levels of IL-6, that is locally produced in response to exercise-induced muscle damage, and the increased creatine kinase level, but it is not a typical finding in HLH.

The immediate aim of HLH treatment is the suppression of the increased inflammatory response and control of cell proliferation using immunosuppressive or immunomodulatory agents and cytotoxic drugs [4]. The treatment differs in children and adults and depends on the underlying disease, the presence of a trigger and severity of symptoms. Chemotherapy using dexamethasone, cyclosporin, and etoposide is used for severe, particularly familial and EBV-associated haemophagocytic syndrome cases [1, 4]. The presented patient was sucessfully treated by corticosteroids and cyclosporin A which corresponds to the expected clinical course of secondary HLH.

Conclusion

The case we reported illustrates the difficulties in distinguishing between primary and secondary HLH. This is very important since the preferred therapeutic approach in these two forms of the disease is quite different. Adult cases with HLH may be associated with „mild“ mutations in the primary HLH-related gene (with „weaker“ phenotypic expression), predisposing to disease initiation upon the action of common trigger(s), thus resulting in clinically secondary HLH. Further studies are needed to clarify the significance of particular gene mutations in the pathogenesis of HLH and to determine optimal therapeutic modalities in different patients suffering from HLH. This appears to be particularly true for those with “atypical” presentations of what is still generally classified as „secondary“ HLH.

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R E F E R E N C E S

1. *Verbsky JW, Grossman WJ.* Hemophagocytic lymphohistiocytosis: Diagnosis, pathophysiology, treatment, and future perspectives. Ann Med 2006; 38(1): 20−31.
2. *Risma K, Jordan MB.* Hemophagocytic lymphohistiocytosis: Updates and evolving concepts. Curr Opin Pediatr 2012; 24(1): 9−15.
3. *Janka GE.* Hemophagocytic syndromes. Blood Rev 2007; 21(5): 245−53.
4. *Henter J, Horne A, Aricó M, Egeler MR, Filipovich AH, Imashuku S,* et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48(2): 124−31.
5. *zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI,* et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet 2005; 14(6): 827−34.
6. *Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J,* et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. Am J Hum Genet 2009; 85(4): 482−92.
7. *Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C.* Infections associated with haemophagocytic syndrome. Lancet Infect Dis 2007; 7(12): 814−22.
8. *Jordan MB, Filipovich AH.* Hematopoietic cell transplantation for hemophagocytic lymphohistocytosis a jorney of thousand miles begins with single (big) step. Bone Marrow Transplantation 2008; 42: 433–7.
9. *Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K,* et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. Haematologica 2010; 95(12): 2080−7.
10. *Janka GE.* Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr 2007; 166(2): 95−109.
11. *Pedersen BK, Bruunsgaard H, Jensen M, Toft AD, Hansen H, Ostrowski K.* Exercise and the immune system: Influence of nutrition and ageing. J Sci Med Sport 1999; 2(3): 234−52.
12. *Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA, Pedersen BK.* Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 1997; 499 (Pt 3): 833−41.

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CASE REPORT

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Pulmonary veins isolation in a patient with atrial fibrillation and pronounced vagal response: Is it enough?

Izolacija plućnih vena kod bolesnika sa fibrilacijom pretkomora i naglašenim vagalnim odgovorom: Da li je to dovoljno?

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Abstract

Introduction. Pulmonary vein isolation (PVI) by antral circumferential ablation is the standard procedure for patients with symptomatic and drug-refractory paroxysmal atrial fibrillation (AF). In some patients addition of ganglionated plexi (GP) modification in anatomic locations to PVI confers significantly better outcomes than PVI alone. **Case report.** We reported a patient with paroxysmal, symptomatic AF and severe bradycardia a month prior to ablation. The patient was treated with antiarrhythmic drugs without success. Because of severe bradicardia the patient was implanted with a temporary pace maker two days before PVI. During PVI the decision was made to also do a modification of the left GP. Three months after the procedure the patients was in stable sinus rhythm without any symptoms. **Conclusion.** In selected patients with paroxysmal AF and pronounced vagal response PVI by circumferential antral ablation combined with GP modification during single ablation procedure can produce higher success rates than PVI or GP ablation alone.

Key words:
atrial fibrillation; pulmonary veins; catheter ablation; vagotomy.

Apstrakt

Uvod. Izolacija plućnih vena (PVI) antrumskom cirkumferentnom ablacijom je standardna metoda za bolesnike sa simptomatskom i na lekove refraktornom atrijalnom fibrilacijom (AF). Kod pojedinih bolesnika dopunska modifikacija anatomskih lokacija autonomnih gangliona *ganglionated plexi* (GP) dovodi do značajno boljeg ishoda od onih kojima je učinjena samo PVI. **Prikaz bolesnika.** Prikazali smo bolesnika sa paroksizmalnom AF i epizodama teške bradikardije koje je imao u poslednjih mesec dana. Bolesnik je lečen antiaritmijskom terapijom bez značajnijeg uspeha. Zbog teške bradikardije bolesniku je implantiran privremeni vodič srčanog ritma dva dana pre elektrofiziološkog ispitivanja i radiofrekventne ablacije. Tokom radiofrekventne ablacije zbog izraženog vagalnog odgovora odlučeno je da se učini i modifikacija anatomskih lokacija GP sa leve strane. Tri meseca kasnije bolesnik je bio u stabilnom sinusnom ritmu i bez simptoma. **Zaključak.** Kod pojedinih bolesnika sa paroksizmalnom AF i naglašenim vagalnim odgovorom, PVI cirkumferentnom antrumskom ablacijom udruženom sa modifikacijom GP, može imati veći uspeh od PVI ili modifikacije GP samostalno.

Ključne reči:
fibrilacija pretkomora; vv. pulmonales; ablacija preko katetera; vagotomija.

Introduction

The most common sustained arrhythmia is atrial fibrillation (AF) and the main concern is that this arrhythmia is associated with significant morbidity and mortality [1]. Many mechanisms have been considered to contribute to the creation of AF, among them the autonomic nervous system (ANS) plays an important role, because in general opinion AF results from the interplay between trigger, substrate and ANS in each patient [1]. The ANS of the heart consists of extrinsic and intrinsic ganglia [2]. The vagus nerve with parasympathetic components is the part of extrinsic cardiac nervous system. The sympathetic components beside the vagus nerve originate primarily from the cervical spinal cord [3]. The mechanisms by which autonomic activation is arrhythmogenic are complex and different for specific arrhythmias. In AF simultaneous sympathetic and parasympathetic activations are the most common trigger. In

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contrast to some other arrhythmias, like ventricular tachycardia, where sympathetic activation is proarrhythmic, parasympathetic activation is antiarrhythmic. PVI is the standard catheter ablation procedure for patients with recurrent, symptomatic or drug-refractory paroxysmal AF. Adjunctive ablation such as performing linear lesions, ablating complex fractionated atrial electrograms or ganglionated plexi (GP) are still used only for improving procedural efficacy in selected patients [4].

Case report

A 37-year old man with severe sinus bradycardia (pulse under 40/min), was admitted to our Clinic, complaining to headache, dizziness and postural instability. One month prior to admission, the patient was admitted to another hospital because of paroxysmal AF and bradycardia. He was treated briefly with antiarrhythmics (amiodaron, beta blockers) but due to profound bradycardia, pulse under 35/min, they were discontinued. His CHA_2DS_2-VASc score (Birmingham stroke risk stratification algorithm) was 0, but regarding preparation for the radiofrequency (RF) ablation the patient was commenced onto warfarin therapy. Transesophageal echocardiography was performed to rule out the presence of left atrial or left atrial apendage thrombus. Left ventricular ejection fraction and the left atrial diameter were normal and no significant valvular lesions were found. Subsequent Holter monitoring revealed episodes of short paroxysmal AF not longer than 5 min triggered by atrial premature beats (APBs) with episodes of bradycardia (minimal frequency on Holter was 35/min). On the third hospital day, due to bradycardia, a temporary pace maker was implanted through femoral vein. On the fourth hospital day the patient underwent electrophysiological study. In terms of analgosedation through the right femoral vein a decapolar electrode catheter was inserted and positioned at the distal coronary sinus. The transseptal access was achieved under fluoroscopic guidance applying the standard technique. Intravenous unfractionated heparin was administered till the end of the procedure (a starting dose of 70 IU/kg and then 1,000 IU *per* hour). Then, spiral computed tomografy was performed and the resulting image of the left atrium (LA) was integrated with the electroanatomic map of the LA. PVI was performed using the Ensite Velocity electroanatomic mapping system (St Jude Medical, Minnesota, USA). First RF application in the posterosuperior part of circumference arund left veins produced profound bradycardia which was then followed by AF initiation. Ablation was carried out at the antral part of all four pulmonary veins (PVs). They were successfully isolated and sinus rhythm was restored spontaneously (Figure 1). After that, ablation was carried out near the areas of anatomic location of GP (Figure 2) As an endpoint for GP ablation we selected abolition of inducibi-

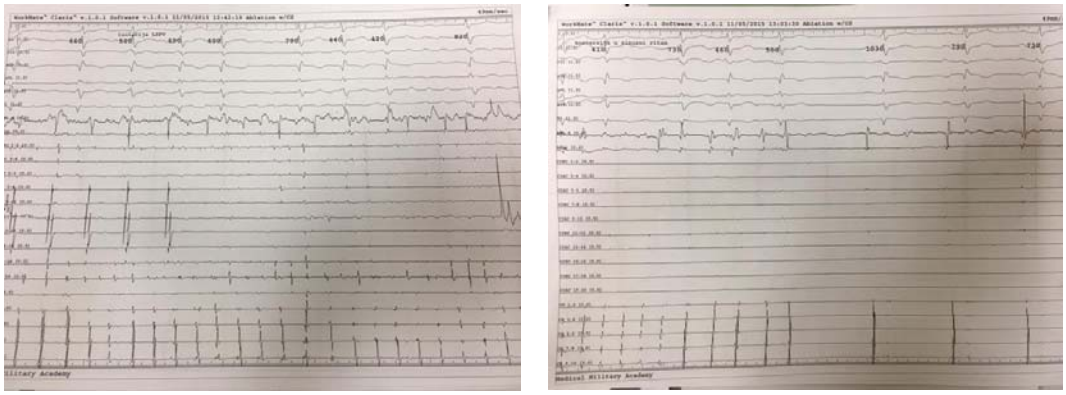

Fig. 1 – Spontaneous termination of atrial fibrillation after ablation of all four pulmonary veins.

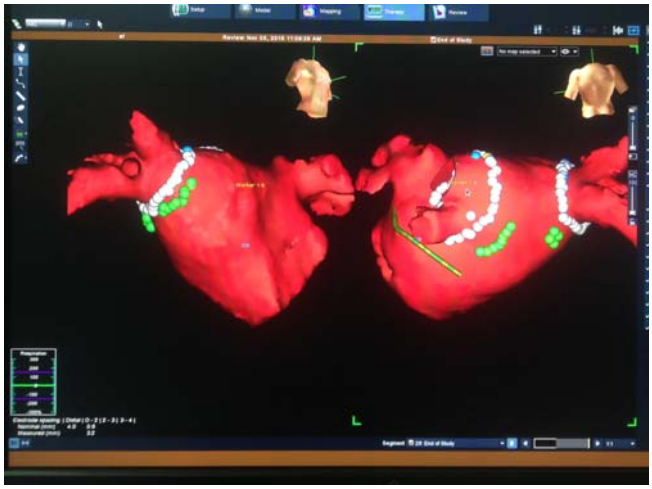

Fig. 2 – Ablation lines shown in white represent pulmonary vein isolation and the lines in green the location of ganglionated plexi ablations.

le vagal response (induction of AF and/or bradicardia and/or atrioventricular (AV) block. Presumed locations of the four major left atrial GP were ablated outside the junctions of PVs and LA at the following sites: left superior GP (SLGP), left inferior GP (ILGP), right anterior GP (ARGP) and right inferior GP (IRGP) and ligament of Marshall [5]. The procedure was performed without complications. During a 3-month follow-up period the patient was in stable sinus rhythm without episodes of atrial fibrillation or sinus bradycardia.

Discussion

In published studies the success rate of GP ablation alone was not significantly superior to pulmonary vein isolation alone, but GP ablation plus PVI significantly increased freedom from AF after a combine procedure both in paroxysmal and persistent AF [6–13]. Regarding pronounced vagal response in the presented patient the decision was made to do PVI plus GP ablation [6]. We decided to ablate the four major left atrial GP according to their anatomic locations. We did not use GP ablation guided by high frequency stimulation (HFS) because recent studies demonstrated that the outcome was inferior to that guided by the anatomic locations of the GP [7]. PVI by antral circumferential ablation eliminates the triggers in the PV antrum and ensures that PV firing cannot conduct into the atrium to initiate AF but at the same time during conventional PVI we also transected the SLGP, the ligament of Marshall, ARGP, and a part of the IRGP, as well as many small clusters of autonomic ganglia and nerves localised around PV. More complete autonomic denervation by GP ablation in combination with PVI can be an additional step forward to beter outcome. Another possibility is that ablation in the GP area also results in elimination of the complex electrical activity located at parts of the LA localised near GP or PV antrum [12]. Regarding fractionated electrograms some studies show that they are usually found in the areas of GP and that is consistent with the possibility that GP ablation may target both autonomic neural elements and fractionated electrograms [4]. Pacemaker implantation in the patient with AF because sinus node disfunction for some time may not be clinically apparent until conversion to sinus rhythm [14]. The patient presented here had a temporary pace maker implanted before ablation and during a follow-up period was in stable sinus rhytm without indication for pace maker. The risk for permanent pace maker implantation after ablation is similar to cardioversion, suggesting that patients require pacing due to a common underlying electrophysiologic substrate, rather than the ablation itself [14, 15]. Longer ablation time and large number of RF application delivered have a higher risk for complications and ablation-induced proarrhythmia, but we did not have such problems in the presented patient [16].

Conclusion

PVI by circumferential antral ablation with GP modification performed in a single ablation procedure in selected patients with paroxysmal AF and prononced vagal response has higher success rates than PVI or GP ablation alone. A short follow-up period in the presented patient is the major disadvantage regarding this conclusion. The long-term benefit and risk in patients with PVI and GP modification ablation deserve further evaluation.

REFERENCES

1. *Calkins H, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen SA* et al. 2012 HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design. Europace 2012; 14: 528–606.
2. *Chen PS, Chen LS, Fishbein MC, Lin SF, Nattel S.* Role of the autonomic nervous system in atrial fibrillation: pathophysiology and therapy. Circ Res 2014; 114(9): 1500−15.
3. *Linz D, Ukena C, Mahfoud F, Neuberger HR, Böhm M.* Atrial autonomic innervation: a target for interventional antiarrhythmic therapy? J Am Coll Cardiol 2014; 63(3): 215−24.
4. *Pappone C, Santinelli V, Manguso F, Vicedomini G, Gugliotta F, Augello G,* et al. Pulmonary vein denervation enhances long-term benefit after circumferential ablation for paroxysmal atrial fibrillation. Circulation 2004; 109(3): 327−34.
5. *Po SS, Nakagawa H, Jackman WM.* Localization of left atrial ganglionated plexi in patients with atrial fibrillation. J Cardiovasc Electrophysiol 2009; 20(10): 1186−9.
6. *Verma A, Saliba WI, Lakkireddy D, Burkhardt JD, Cummings JE, Wazni OM,* et al. Vagal responses induced by endocardial left atrial autonomic ganglion stimulation before and after pulmonary vein antrum isolation for atrial fibrillation. Heart Rhythm 2007; 4(9): 1177–82.
7. *Katritsis D, Giazitzoglou E, Sougiannis D, Goumas N, Paxinos G, Camm AJ.* Anatomic approach for ganglionic plexi ablation in patients with paroxysmal atrial fibrillation. Am J Cardiol 2008; 102(3): 330–4.
8. *Danik S, Neuzil P, d'Avila A, Malchano ZJ, Kralovec S, Ruskin JN* et al. Evaluation of catheter ablation of periatrial ganglionic plexi in patients with atrial fibrillation. Am J Cardiol 2008; 102(5): 578–83.
9. *Pokushalov E, Romanov A, Artyomenko S, Turov A, Shirokova N, Katritsis DG.* Left atrial ablation at the anatomic areas of ganglionated plexi for paroxysmal atrial fibrillation. Pacing Clin Electrophysiol 2010; 33(10): 1231−8.
10. *Mikhaylov E, Kanidieva A, Sviridova N, Abramov M, Gureev S, Szili-Torok T,* et al. Outcome of anatomic ganglionated plexi ablation to treat paroxysmal atrial fibrillation: a 3- year follow-up study. Europace 2011; 13(3): 362–70.
11. *Calo L, Rebecchi M, Sciarra L, De Luca L, Fagagnini A, Zuccaro LM,* et al. Catheter ablation of right atrial ganglionated plexi in patients with vagal paroxysmal atrial fibrillation. Circ Arrhythm Electrophysiol 2012; 5(1): 22–31.
12. *Zhou Q, Hou Y, Yang S.* A meta-analysis of the comparative efficacy of ablation for atrial fibrillation with and without ablation of the ganglionated plexi. Pacing Clin Electrophysiol 2011; 34(12): 1687–94.
13. *Pokushalov E, Romanov A, Shugayev P, Artyomenko S, Shirokova N, Turov A,* et al. Selective ganglionated plexi ablation for paroxysmal atrial fibrillation. Heart Rhythm 2009; 6(9): 1257–64.
14. *Deshmukh AJ, Yao X, Schilz S, Van Houten H, Sangaralingham LR, Asirvatham SJ,* et al. Pacemaker implantation after catheter ablation for atrial fibrillation. J Interv Card Electrophysiol 2016; 45(1): 99−105.

15. *Katritsis DG, Pokushalov E, Romanov A, Giazitzoglou E, Siontis GC, Po SS,* et al. Autonomic denervation added to pulmonary vein isolation for paroxysmalatrial fibrillation: a randomized clinical trial. J Am Coll Cardiol 2013; 62(24): 2318−25.
16. *Osman F, Kundu S, Tuan J, Jeilan M, Stafford PJ, Ng GA.* Ganglionic plexus ablation during pulmonary vein isolation—predisposing to ventricular arrhythmias? Indian Pacing Electrophysiol J 2010; 10(2): 104−7.

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I N M E M O R I A M

prof. dr BOGDAN BOŠKOVIĆ
pukovnik u penziji
(1931–2017)

Dana 5. aprila ove godine, iznenadila nas je smrt pukovnika u penziji, profesora, doktora medicinskih nauka Bogdana Boškovića, izuzetno cenjenog i dragog kolege, a za one sa kojima je bio blizak - našeg Boce. Iznenađeni smo jer po mentalnoj i fizičkoj vitalnosti, poletu i entuzijazmu, niko nije očekivao, čak ni pretpostavljao, da će nas smrt tako naprasno i nenadano odvojiti od njega.

Stari Latini su nam ostavili glasovito pravilo o pristojnom ponašanju kada se govori o pokojnicima: *De mortius nihil nisi bene* - O mrtvima sve najbolje. Ta sentenca se često koristi s upadljivim preterivanjem, ponekad bez ikakvih kriterijuma. Međutim, ovog puta ja imam drugačiji problem - kako da se ono što treba reći o profesoru Bogdanu Boškoviću, kao suštinsku istinu, ne izgubi u sivilu puke, običajne konvencije.

Prof. dr Bogdan Bošković rođen je 17. septembra 1931.. godine u selu Konjuh kod Kruševca. Osnovnu školu je završio u rodnom mestu, a gimnaziju u Kruševcu. Završio je dva fakulteta: farmaceutski u Zagrebu, 1956. godine, i medicinski u Sarajevu, 1964. godine. Magistrirao je 1961, specijalizaciju iz toksikologije bojnih otrova završio je 1963, a doktorsku disertaciju pod naslovom „Prilog mehanizmu delovanja reaktivatora holinesteraze“ odbranio je na Medicinskom fakultetu u Sarajevu 1968. godine. Na stručnom usavršavanju u oblasti farmakologije boravio je u Velikoj Britaniji u dva navrata, 1964. i 1969. godine.

Od kraja 1964. do penzionisanja 1993. godine radio je u Vojnotehničkom institutu (VTI) u Beogradu gde je prošao sve faze napredovanja u oficirskoj hijerarhiji, struci, nauci i rukovođenju: od kapetana do pukovnika, od istraživača do naučnog savetnika i redovnog profesora, od rukovodioca grupe za nuklearno-hemijsko-biološku zaštitu, preko načelnika odseka i odeljenja, do načelnika Sektora za medicinsku zaštitu i inženjersko obezbeđenje. Tokom rada u VTI-u, pod njegovim rukovodstvom vršena su opsežna istraživanja iz različitih oblasti vojne toksikologije koja su imala za cilj razjašnjenje mehanizama toksičnosti najvažnijih grupa bojnih otrova (nervni bojni otrovi, plikavci, cijanidi) i pronalaženje njihovih najefikasnijih antidota i sredstava zaštite. Istovremeno, vršena su istraživanja o mogućnostima zaštite od pojedinih bioloških agenasa, kao i istraživanja hemijskih radioprotektora koji bi bili u stanju sprečiti, odnosno ublažiti radijacionu povredu. Rezultati ovih istraživanja publikovani su u najpoznatijim svetskim časopisima, zahvaljujući kojima je naša vojna toksikologije bila prepoznata kao jedna od vodećih u svetu. Godinama posle, na svetskim toksikološkim skupovima često smo od kolega iz drugih zemalja slušali kako s poštovanjem govore o toj čuvenoj „Beogradskoj toksikološkoj školi“.

Godine 1981. izabran je za redovnog profesora farmakologije i toksikologije u Vojnomedicinskoj akademiji (VMA). Od 1986. do 1990. godine bio je redovni profesor na poslediplomskim studijama na Medicinskom fakultetu u Tuzli, a od 1998. godine držao je po pozivu predavanja iz farmakologije i toksikologije studentima Medicinskog fakulteta u Foči.

Bio je član Srpskog lekarskog društva, Društva farmakologa Jugoslavije (kasnije Srpskog farmakološkog društva), Udruženja toksikologa Jugoslavije (kasnije Srbije), Farmaceutskog društva Srbije, glavni i odgovorni urednik i jedan od osnivača dva domaća stručna časopisa na engleskom jeziku, član uredništva i recenzent više domaćih i međunarodnih medicinskih časopisa uključujući i Vojnosanitetski pregled.

Godine 1971. izabran je za člana Britanskog farmakološkog društva, a 1996. godine za člana Nemačkog društva za farmakologiju i toksikologiju. Za eksperta Svetske zdravstvene organizacije za toksikologiju pesticida izabran je 1972. godine.

Profesor Bošković je bio član Saveta za naučni rad u oružanim snagama bivše Jugoslavije i predsednik Komisije za naučne kadrove tog saveta. Više godina je bio rukovodilac Naučnog projekta Vlade Socijalističke Republike Srbije pod nazivom „Zaštita hrane od zagađivanja i zdravstveni aspekti zagađivanja“. Bio je član Naučnog društva Srbije i predsednik Savezne komisije za dijagnostiku i terapiju u bivšoj SFRJ.

Objavio je oko 400 naučnih i stručnih radova, većinu u prestižnim međunarodnim časopisima, koji su citirani više od 750 puta. Autor je monografije "Pesticidi - toksikologija i terapija trovanja" i koautor poglavlja u tri domaća udžbenika i u dve inostrane monografije. Bio je mentor i član za odbranu 54 magistarska rada i 68 doktorskih disertacija.

Održao je više predavanja po pozivu na prestižnim univerzitetima u SAD i Velikoj Britaniji i učestvovao, kao pozvani predavač, na šest međunarodnih naučnih skupova iz oblasti toksikologije i farmakologije.

Nastavničkim radom, mentorstvom i učešćem u komisijama za izradu i odbranu magistarskih teza i doktorskih disertacija profesor Bošković je značajno doprineo razvoju medicinskih fakulteta u Beogradu, Banjaluci, Nišu, Novom Sadu, Prištini, Sarajevu, Foči, Rijeci, Tuzli i Zagrebu, kao i razvoju naučnoistraživačkog podmlatka u VTI i VMA u Beogradu.

Profesor Bogdan Bošković dao je značajan doprinos razvoju eksperimentalne i primenjene toksikologije i farmakologije na svetskom nivou, posebno u oblasti prevencije i terapije akutnih trovanja organofosfornim jedinjenjima (OFJ) iz grupe nervnih bojnih otrova i organofosfornih insekticida. Njegovo razjašnjenje mehanizma toksičnog dejstva nervnog bojnog otrova somana doprinelo je da se u kliničku praksu uvede do tada zaboravljeni i potisnuti reaktivator acetilholinesteraze, HI-6, što je znatno poboljšalo efikasnost lečenja ovih trovanja. Na osnovu rezultata tima eksperata na čelu sa profesorom Boškovićem u kliničku praksu u Jugoslaviji uveden je HI-6 kao antidot za lečenje akutnih trovanja OFJ, a nakon toga ovaj lek je ušao u obavezan sastav ratnog sanitetskog kompleta u armijama tadašnje SFRJ, SAD, Kanade i Švedske. Ne postoji nijedna ozbiljnija publikacija koja se bavi problemom lečenja akutnih trovanja OFJ, uključujući i nervne bojne otrove, a da se u njoj ne citiraju istraživanja i rezultati profesora Boškovića i njegovih saradnika iz Medicinskog odeljenja VTI-a u Beogradu. Ova istraživanja su našla praktičnu primenu u lečenju akutnih trovanja organofosfornim insekticidima koji se koriste u poljoprivredi, šumarstvu, humanoj i veterinarskoj medicini.

Osim istraživanja iz domena vojne toksikologije, svakako treba spomenuti ona koja su su se odnosila na ispitivanja mehanizama dejstva lekova i otrova na različitim eksperimentalnim *in vitro* i *in vivo* modelima, proučavanje terapije Parkinsonove bolesti, terapije eksperimentalno izazvane kongenitalne miotonije, mijastenije gravis, konvulzivnih stanja i terapije bola. Kao rezultat ovih poslednjih istraživanja razvijen je i u kliničku praksu uveden analgetik na bazi kombinacije antiepileptika karbamazepina i nesteroidnog antiinflamatornog leka etodolaka, koji je pokazao izvanrednu efikasnostt u terapiji umerenih do jakih bolova različite etiologije. Taj lek je bio i registrovan za upotrebu u našoj zemlji pod zaštićenim nazivom Novokomb® (1995-2001), ali proizvođač nije obnovio njegovu registraciju jer je u međuvremenu prešao na proizvodnju dijetetskih suplemenata. Za istraživanja i razvoj ovog leka prof. Bošković je nagrađen Plaketom Nikola Tesla na jednom od skupova pronalazača u našoj zemlji.

Pukovnik prof. dr Bogdan Bošković nosilac je šest visokih domaćih vojnih odlikovanja, kao i međunarodnog ordena International Order of Merit (IOM) koji je 2000. godine dobio od Međunarodnog biografskog centra u Kembridžu, Velika Britanija. Biografski podaci o profesoru Bogdanu Boškoviću mogu se pronaći u „Vojnom leksikonu“ (1981), ediciji „Ko je ko u Srbiji“ (1996), „Međunarodnom leksikonu prestižnih ličnosti" Američkog biografskog instituta (1998), publikaciji „2000 istaknutih intelektualaca 21. veka“ Međunarodnog biografskog centra u Kembridžu (2003) i biografskom leksikonu „Poznati srpski lekari“ (2005).

Ako bih sebi dao za pravo da se kritički osvrnem na profesionalni put profesora Bogdana Boškovića, bio bih u ozbiljnoj dilemi: da li je bio briljantniji kao istraživač-naučnik, publicista ili pedagog. Posedovao je osobine vrhunskog naučnika i istraživača: kompetentnost, lično i akademsko poštenje, inicijativnost i sistematičnost, ali i ne manje važne odrednice: pronicljivost, upornost, oprez i odmerenost u zaključivanju. Uvek su mu vodilje bile dve univerzalne mudrosti i njih se držao do kraja života: jedna latinska „Non dies sine linea“ („Nijedan dan bez poteza“), a druga srpska, narodna „Tri put meri, jednom seci“. Nije bio sklon avanturizmu niti senzacionalizmu. Sve je procenjivao realno, sa dozom kritičnosti ozbiljnog naučnika: i svet oko sebe, i ljude kao individue i ličnosti, i društvene tokove, i stanje u nauci i struci, i sopstvene rezultate i svakodnevne događaja. Na osnovu realnih procena, postavljao je ostvarive zahteve i ciljeve, vršio je temeljne i sistematične pripreme, pratio svaku fazu istraživanja ili bilo kod drugog posla kojim se bavio, minuciozno zapažao greške i napretke, uspehe i nedostatke, nalazio načine za dodatne motive, pomerao granice postojećih znanja.

Mi, vojni toksikolozi i farmakolozi, nismo imali potrebu da se obraćamo za stručnu pomoć kolegama u Betezdi, Volter Ridu, Vojnomedicinskoj akademiji u Lenjingradu, Karolinskoj bolnici u Štokholmu, Institutu za toksikologiju u Minhenu, Svetskoj zdravstvenoj organizaciji u Ženevi, Imperijal koledžu u Londonu. Dovoljno je bilo da potražimo prof. Bogdana Boškovića i da iznesemo problem koji imamo. Dešavalo se da mi ni ne ispričamo da kraja svoje dileme, a da profesor nastavi priču, postavi pitanje i počne da daje odgovor. To je važilo za sve oblasti eksperimetnalne i kliničke toksikologije, za bazičnu i kliničku farmakologiju, za farmaciju, za metodologiju naučnog istraživanja i publikovanja i za sve one koji mu se obrate.

Sa mlađima je ravnopravno razgovarao. Dijalog mu je bio omiljena forma komunikacije. Kada bi nepoznati posmatrač pratio samo govor tela dvojice sagovornika, nikada ne bi mogao sa sigurnošću da tvrdi ko je profesor, a ko je student. Međutim, sve bi mu bivalo jasno kada bi čuo živu reč.

Razgovori sa profesorom Boškovićem su uvek bili inspirativni, sadržajni, puni njegovih ideja. Ideje je iznosio odmah, na licu mesta, bez zadrške i rezerve, kao da ih je imao odranije pripremljene.

Ovo su samo najosnovniji podaci o profesoru Bogdanu Boškoviću, velikanu nauke i struke, doajenu jugoslovenske i srpske toksikologije i farmakologije, čoveku koji je ugradio svoj život u temeljne vrednosti društva, medicine, kao svog profesionalnog izbora, i svoje porodice.

Nadam se da smo mi, toksikolozi i farmakolozi, svesni koliko smo bili privilegovani i počastvovani što smo imali priliku da živimo i radimo sa pukovnikom prof. dr Bogdanom Boškovićem. Smatram da sadašnje generacije toksikologa i farmakologa treba ozbiljno, još jednom da izuče sve ono što je profesor uradio, napisao i objavio, da nastave sa radom i realizuju neke od njegovih ideja i projekata, koje zbog kratkoće života i neumitnih prirodnih zakona nije stigao sam da dovrši.

Odlazak profesora Boškovića gubitak je za sve, pre svega za njegovu porodica, ali i za nas, njegove saradnike, kolege, prijatelje i poštovaoce. Zahvalni smo mu i ponosni što smo bili deo njegovog vremena, sveta i dela.

Neka je večna slava i hvala dragom i poštovanom pukovniku prof. dr Bogdanu Boškoviću!

dr sc. med. Veljko Todorović
brigadni general u penziji

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

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

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

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

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

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

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

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

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

Tabele

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

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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