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OCCUPATIONAL MEDICINE - THEN AND NOW: WHERE WE COULD GO FROM HERE

MEDICINA RADA NEKAD I SAD: U KOM PRAVCU DALJE

Karen BELKIĆ[1-3] and Olesja NEDIĆ[4]

Summary
Occupational medicine has a long-standing history in the region of the former Yugoslavia with seminal contributions to the theory and practice of this discipline. This tradition should be expanded to incorporate psychosocial stressors. We review the sociological work stress models and empirical evidence gleaned thereby, and then the occupational stressor index, an additive burden model developed from a cognitive ergonomics perspective. In numerous studies, the occupational stressor index is significantly associated with risk behaviors: smoking, obesity and sedentariness and clinical outcomes: hypertension, ischemic heart disease, dyslipidemia and type 2 diabetes. The occupational stressor index characterizes the work conditions of physicians including surgeons and anesthesiologists; professional drivers and other groups at elevated risk for stress-related disorders. Much of these empirical data are from this region. Work-stress related health disorders are a major public health problem, with enormous human and economic costs. A more proactive role for physicians is needed vis-à-vis our working environment and that of patients. We physicians face a heavy job stressor burden strongly implicated with adverse health outcomes. The challenge is to identify effective strategies to lower the risk of work-stressor related illness. The critical gap is the lack of evidence-based guidelines. Intervention studies are needed in which job stressors are ameliorated as a therapeutic/preventive modality; the logical starting point is within our own profession. We also suggest how the relevant clinical competence could be enhanced. Alongside clinical enhancement should be the full restoration of physician empowerment to implement work-related recommendations. A participatory action research perspective by physicians for physicians and for our patients is needed.
Key words: Occupational Medicine; Workload; Stress, Psychological; Occupational Diseases; Health Promotion; Risk Factors

Sažetak
Medicina rada ima dugogodišnju tradiciju u regionu bivše Jugoslavije sa originalnim doprinosima u teoriji i praksi, koje bi trebalo proširivati uključivanjem psihosocijalnih stresora. Prikazani su sociološki modeli utvrđivanja postojanja profesionalnog stresa i dokazi iz empirijskih istraživanja. Opisan je i novi model indeksa profesionalnog stresa (*Occupational Stressor Index*), potekao iz perspektive kognitivne ergonomije. U brojnim studijama indeksom profesionalnog stresa utvrđena je značajna povezanost profesionalnog stresa sa rizičnim ponašanjima: pušenje, gojaznost, sedenternost i kliničkim ishodima: hipertenzija, ishemijska bolest srca, dislipidemija i dijabetes tipa 2. Indeks profesionalnog stresa sveobuhvatno opisuje sve uslove rada lekara, uključujući hirurge i anesteziologe, profesionalnih vozača i drugih profesija sa povišenim rizikom za pojavu zdravstvenih poremećaja povezanih sa stresom na radu. To je empirijski utvrđeno i u ovom regionu. Zdravstveni poremećaji u vezi sa stresom na radu su veliki javnozdravstveni problem, sa ogromnim ljudskim i ekonomskim posledicama. Ukazano je na potrebu proaktivnije uloge lekara naspram našeg radnog okruženja i pacijenata. Lekari se suočavaju sa teškim zdravstvenim ishodima kao posledicom postojanja velikog profesionalnog opterećenja. Izazov je identifikovati efektivne strategije kojima bi se smanjili rizici od pojave bolesti povezanih sa stresom na radu. Kritični raskorak je u nedostatku smernica zasnovanih na dokazima. Potrebne su interventne studije u kojima bi se na utvrđene profesionalne stresore terapijski/preventivno delovalo; logično polazište je naša vlastita profesija. Takođe sugerišemo kako se relevantne kliničke kompetencije mogu pojačati. Zajedno sa poboljšanjem kliničkog znanja trebalo bi poboljšati osposobljenost lekara u sprovođenju preporuka u vezi sa radom i radnim okruženjem. Potrebna je perspektiva aktivnog učešća u istraživanju lekara za lekare i lekara za pacijente.
Ključne reči: Medicina rada; Izgaranje na poslu; Psihološki stres; Profesionalna oboljenja; Promocija zdravlja; Faktori rizika

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Abbreviations

ACVD – acquired cardiovascular disorders
BMI – body mass index
CI – confidence interval
FRP – favorable risk profile
OR – odds ratio
OSI – Occupational Stressor Index
RTW – return to work

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This paper is dedicated to the memory of Professor Velimir Potkonjak

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Rich History of Occupational Medicine in the Region of the Former Yugoslavia

Occupational medicine has a rich and long-standing history in this region of the world, having made seminal, multi-faceted contributions to the theory and practice of this discipline for many decades. An example is the comprehensive textbook [1], which includes the contributions of a total of eighty-nine authors. It is particularly noteworthy that therein physician specialists in disciplines such as internal medicine, ophthalmology, otorhinolaryngology, dermatology, as well as subspecialists in pulmonary medicine, infectious disease, inter alia, devoted their expertise towards evaluating the relation between the work environment and health. From this basis, emerged sophisticated approaches for assessing work fitness and optimizing worker protection, including functional diagnostic laboratories for ergo-ophthalmology and for evaluating pulmonary dynamics in relation to occupational exposures, to name a few. The importance of the contributions from this region to the field of occupational medicine has come to be particularly appreciated in the recent period, for example at the Workshop on Healthy Work for Health Workers [2].

Need for Expansion to Consider Psychosocial Stressors

As traditionally the case for occupational medicine, the focus of the above-cited activities and endeavors has mainly been upon physical and chemical exposures. However, with technological advances, jobs characterized purely by heavy physical demands and physical/chemical exposures have become progressively less common. New types of work-related challenges and burdens mainly affecting the higher nervous system (i.e. psychosocial stressors) are increasingly encountered [3]. As emphasized by Hu and Speizer [4], it is vital to identify job-related and other environmental hazards that contribute to a given disease process. These authors aptly noted: ”physicians commonly treat the sequelae of such disease in the practice of medicine; however, unless the underlying connection with hazardous exposures is identified and mitigated, treatment of the manifestations rather than the cause at best only ameliorates the condition. At worst, the neglect of hazardous exposures may lead to both failure of treatment and failure to recognize a public health problem with wide significance [p 19].”

Assessment of the Psychosocial Work Environment

With regard to psychosocial stressors, theoretical conceptualization, modeling and measurement are critical challenges. Evidence which relies solely upon subjective perceptions, such as e.g. dissatisfaction with one’s job, is insufficient to motivate the policy decisions needed to redress hazards related to psychosocial exposures. It is clear that this evidence is much more difficult to accumulate compared to that for physical or chemical exposures, where the cause of injury is often clearly work-related.

Sociological Models and Empirical Evidence Gleaned Thereby

In 1979, a major breakthrough was made with the introduction of the Job Strain Model [5] grounded in sociological theory. This model was developed for work environments in which stressors are: ”chronic, not initially life-threatening and the product of sophisticated human organizational decision making. In decision making the controllability of the stressor is critical, and it becomes more important as increasingly complex and integrated social organizations develop, with ever more complex limitations on individual behavior” (p. 78) [6].

The model has two components: psychological demands, and a combined measure of task control and skill use, termed decision latitude. Job strain occurs when there is psychological overload and at the same time, the person lacks control over his or her work environment.

A complementary sociologically-based model, Effort-Reward Imbalance [7, 8], was subsequently introduced, in which the focus is on a lack of reciprocity between the effort made and rewards received. The latter include financial rewards, appreciation, opportunities for career advancement and job security. According to the latter model, efforts can be extrinsic (job demands and obligations) as well as intrinsic (over-commitment to work).

Heavily based upon these sociological models, etiologic research has demonstrated a strong relationship between workplace stressors and adverse health outcomes, notably cardiovascular disease [3, 9] and mental health disorders [10, 11].

Occupational Stressor Index: Comprehensive Model based on Cognitive Ergonomics

Considering the success of this line of research, together with the worsening of work conditions, which is occurring worldwide, it becomes incumbent upon us to sharpen our tools, so that efforts to create more flexible and healthier work environments become maximally effective [12]. Vital to these efforts is to incorporate a cognitive ergonomics perspective, one which addresses how human beings actually process information, make decisions, and carry out actions [13]. The Occupational Stressor Index (OSI) [11, 12, 14] is an additive burden model developed from this cognitive ergonomics perspective and which also incorporates key aspects of the Job Strain and Effort–Reward Imbalance models. The OSI analyzes work in relation to demands on mental resources and how these demands are controlled by the individual, consistent with the Energy Regulation Theory [15]. This theory shows that the two job-strain dimensions are closely coupled, such that with sufficient decision latitude, a person can modulate even fairly onerous, although not overwhelming, psychological workload to meet his or her needs and capacities. At the same time, it becomes critical to rigorously define and guard against exposure to overwhelming job demands. With the help of cognitive ergonomics, the burden of work processes upon the central nervous system can be described in a relatively objective way [12, 14].

Within the OSI, the work environment is viewed as a whole, including task-level issues, work schedule, physical and chemical exposures as well as broader organizational factors that can all contribute to the total stressor burden. In other words, the OSI provides a comprehensive assessment of an individual's job conditions, akin to and compatible with the clinical approach of taking a complete occupational history, with the added benefit of quantitative information and normative data. Of particular note is the inclusion of key stressor dimensions such as threat avoidant vigilance [16] that are missing from the sociological models. Without consideration of these relatively "silent" factors such as the need to maintain high levels of vigilance to avoid potentially disastrous consequences, the stressor burden of our own profession, that of nurses and other health professionals, airline pilots, bus drivers, *inter alia*, is substantially underestimated [11, 12]. A version of the updated 2014 OSI model is presented in **Table 1**.

Empirical Studies using the OSI

In a substantial number of published studies [17–27] the total OSI, its aspects and many of the elements were found to be significantly associated with risk behaviors such as smoking, obesity and sedentariness and with clinical outcomes, including arterial hypertension, ischemic heart disease, as well as dyslipidemia, type 2 diabetes, *inter alia*. Moreover, the total OSI scores and OSI profiles help identify and characterize the work conditions of occupational groups such as surgeons, anesthesiologists, other physician categories, as well as urban mass transit operators, long-route truck drivers and other professional driver groups at elevated risk for stress-related disorders. The cited empirical data were obtained in large measure, though by no means exclusively, within this region. The OSI questionnaires, including the most recent 2014 versions, have been validated for use herein, having been prepared via the translation-back-translation method. Permission to use any of the OSI instruments should be obtained from the 1st author. We provide permission free-of-charge for all clinical and research endeavors aimed at improving job conditions and health.

Starting with our Own Profession: Why Physicians?

Physicians who complete the rigorous training and enter the workplace are a highly selected group. Consequently, among our profession, there is a very strong "healthy worker effect", such that it is expected that disease occurrence will be substantially lower than in the general population or even in other occupations. This is a particularly important consideration for stress-related illnesses such as the acquired cardiovascular disorders, as well as mental health disorders [3, 28, 29]. In other words, in training, hiring and retention into most of these highly stressful professions, there is a marked selection of mentally and physically very healthy persons, since such persons are more likely to be productive and adaptable to difficult work situations [29]. Furthermore, physicians are well aware of lifestyle-related and other factors that contribute to or protect against these illnesses.

Risk for Stress-Related Disorders among Physicians despite a "Super-Healthy Worker Effect"

In this light, the strong and consistent evidence that physicians are at increased risk of suicide [30–32] and burnout [33–36] strongly implicates a work-related etiology. Stressors such as harassment/degrading experiences, night shift work, violence from patients and patient suicide, *inter alia*, have been identified as precipitating factors [34, 37–39].

Although evidence is still lacking that physicians are at increased risk for the stress-related or the so-called acquired cardiovascular disorders (ACVD) [40] compared to other occupational groups [41], once hypertension develops, physicians appear to be at high risk for complications. This statement is based upon a 7-year follow-up study of 160 physicians and nurses in Vojvodina

Table 1. Bilingual Version (English-Serbian) of the Occupational Stressor Index version 2014
Tabela 1. *Indeks profesionalnih stresora verzija 2014*

Aspects-Levels *Aspekti-Nivoi*	Underload *Podopterećenje*	High Demand *Visoki zahtevi*	Strictness *Strogost-tačnost*	Time pressure *Spoljašnji vremenski pritisak*	Exposure to noxins *Izloženost noksama*	Avoidance/Symbolic Aversiveness *Averzivnost/Izbegavanje opasnosti*	Conflict/Uncertainty *Konflikti/neizvesnost*
Input *Primanje informacija*	• Homogeneous signals *Istovrsne informacije* • Low frequency of incoming signals *Retko pristizanje novih signala* • Works alone-without need for communication *Radi sam bez potrebe za komunikacijom*	• Several information sources *Više izvora informacija* • Heterogeneous information *Raznorodne informacije* • Heavy burden on visual system *Primarno vizuelno opažanje* • High frequency of incoming signals *Visok tok novih informacija* • 3 sensory modalities *3 čulna nadražaja istovremeno* • Communication essential *Neophodnost komunikacije pri radu*	• Strict requirements for signal detection *Strogi zahtevi za tačnost u detekciji signala*	• No control over speed of incoming signals *Ne kontroliše brzinu pristižućih informacija*	• Glare *Bljesak* • Noise *Buka*	• High level of attention (Serious consequences of momentary lapse) *Visok nivo trajne pažnje/nesagledive posledice momentalnog pada nivoa pažnje* • Visually-disturbing scenes *Izloženost vizuelno uznemirujućim scenama* • Listens to emotionally-disturbing occurrences *Izloženost emocionalno uznemirujućim događajima*	• Signal/noise conflict *Nejasna razlika između šuma i signala* • Signal/signal conflict *Nejasna razlika između različitih signala*
Central Decision-Making *Donošenje odluka*	• Decisions automatic from input *Odluke slede automatski na osnovu primljenih informacija*	• Complex decisions/*Složene odluke* • Complicated decisions *Komplikovane odluke* • Decisions affect work of others/*Odluke utiču na rad drugih* • Rapid decision-making *Donošenje brzih odluka*	• Strict problem-solving strategy *Ograničenja u strategiji rešavanja problema* • Strictly-defined correct decision/*Strogo ograničen broj tačnih odluka*	• Decisions cannot be postponed *Odluke se ne mogu odložiti*		• Serious (potentially fatal) consequences of a wrong decision *Teške (eventualno smrtonosne) posledice pogrešnih odluka*	• Missing information needed for decision/*Nedostatak informacija za donošenje odluka* • Contradictory information *Protivrečne informacije* • Unexpected events change work plan/ *Novi plan rada zbog nepredviđenih događaja*
Output/Task Performance *Izvršavanje zadataka*	• Homogenous tasks *Istovrsni zadaci* • Simple Tasks *Jednostavni zadaci* • Nothing to do (includes waiting time) *Nedovoljan posao - nema ništa da radi (uključujući vreme čekanja)*	• Heterogeneous tasks *Raznorodni zadaci* • Simultaneous task performance *Istovremeno izvršavanje zadataka* • Complex tasks *Složeni zadaci* • Rapid task performance *Brzo izvršavanje zadataka*	• Work must meet a strictly-defined standard *Stroga kontrola rada po pravilima*	• No control over rate of task performance *Nema uticaja na tempo rada*	• Isometric lifting *Dizanje tereta* • Vibration *Vibracije*	• Hazardous task performance *Akutne opasnosti pri radu*	• Conflicting demands *Protivrečni zadaci* Task performance hampered by: *Ometanje rada zbog:* • Extrinsic problems/*Spoljašnjih problema* • Interruptions from people *Prekida od strane saradnika (ljudi)*
General *Opšti*	• Fixed pay *Fiksna plata* • Inadequate pay *Neadekvatna plata* • No chances for upgrade *Nemogućnost napredovanja u karijeri* • Lack of recognition of work *Nedostatak priznanja za rad*	• Piece rate work *Plata po učinku* • Long work hours/*Dugo radno vreme* • Holds 2+ jobs *Honorarni rad* • Lack of rest breaks *Nedostatak pauze u toku rada* • Night shift work/*Noćni/smenski rad* • Lack of paid vacations (including being obliged to work during that time) *Nedostatak plaćenog odmora (uključujući ako radi za vreme plaćenog odmora)*	• Fixed body position/*Fiksiran telesni položaj* • Confined workspace/*Sužen radni prostor* • Lack of autonomous workspace *Nema sopstvenog radnog prostora* • Limited in taking time off from work/ *Ograničene mogućnosti uzimanja slobodnih dana/sati* Low influence over/*Ograničen uticaj na:* • Schedule/*Radni razpored* • Tasks/*Zadatke* • Policy/*Politiku ustanove* • With whom one works/*Izbor saradnika*	• Deadline pressure *Rad vezan za vremenski rok* • Speed-up *Ubrzavanje rada*	• Heat *Visoka temperatura* • Cold *Niska temperatura* • Gases, fumes, dusts *Gasovi, pare, prašine*	• Work Accident *Doživljene povrede na radu* • Witnessed work accident *Svedok povrede na radu* • Work-related litigation/Testifying in court/*Parničenje na sudu* • Suicide occurrence *Samoubistvo u okviru rada* • Lack of functioning emergency system *Nedostatak sistema za slučaj opasnosti*	• Emotionally-charged work atmosphere/*Emocionalno opterećena radna atmosfera-konflikti* • Lack of help with work-related difficulties/*Nedostatak pomoći od kolega* • Opposition to career advancement *Protivljenje unapređenju karijere* • Violations of behavior norms/abuses of power *Kršenje normi ponašnja/zloupotreba vlasti* • No grievance redress/*Nema načina žalbe* • Threat of job loss *Pretnja otpuštanjem* • Job lacks coherence/*Posao bez smisla*

[42]. In comparison to 122 hospital employees without clinical duties, the health professionals had a relative risk = 3.7 (95% confidence interval (CI) = 1.6 - 8.6) for developing cardiovascular or cerebrovascular complications. These findings corroborate the special etiological importance of occupational stressors in the progression from hypertension to ischemic heart disease [43, 44].

In a case-control study [24, 45] applying the OSI among 208 physicians employed at the Novi Sad Clinical Center, the total OSI score was significantly higher for the cases (those with one or more of the ACVD) compared to the control group of physicians. Two dimensions: high demands and threat avoidant vigilance were dominant in showing higher exposure among the cases. The stressors that most consistently and significantly distinguished physicians with ACVD from referents were long work hours, speed-up, and threat of job loss. It was concluded that physicians are a heavily burdened occupational group, and several occupational stressors are significantly associated with case status [24, 45].

Several other studies [17, 18, 46] from the same cohort examined the relationship between work stressors assessed via the OSI and lifestyle-related risk factors for cancer and heart disease (smoking, obesity, sedentariness, and alcohol consumption). It is noteworthy that Novi Sad is a region with a high prevalence of lifestyle-related risk factors for cancer and heart disease. In the first of these studies, focusing upon the 112 participating female physicians [17], the total OSI score and several aspects of occupational stress, notably threat avoidance alone or in combination, showed significant multivariate associations with the lifestyle-related risk factors for cancer and heart disease, as did individual stressors identified by the OSI. The latter included long work hours, restricted problem-solving strategies, insufficient help with clinical difficulties, supervisory responsibility (significant for obesity or sedentariness), and problems hampering patient care (significant for smoking). More recently, a comparison of the participating male and female surgeons/anesthesiologists and the other physicians revealed a significantly higher total OSI score in the former, with night shift work identified as a significant correlate of lifestyle-related risk factors, whereas the total OSI was implicated for the male and female physicians working in non-surgical specialties [18, 46].

A comparative study from the Novi Sad physician cohort was also performed among 35 female physicians with and 74 without clinically-diagnosed hypertension [26]. Adjusting for covariates including body mass index (BMI), having an OSI high demand score above the mean yielded an odds ratio (OR) of 3.14 (95% CI=1.05–9.43) for hypertension. However, overweight physicians *without* diagnosed hypertension were more often current and heavier smokers. The total OSI score was significantly lower among the physicians with the favorable risk profile, defined in that study as not a current smoker and without diagnosed hypertension. The most powerful multivariate model for favorable risk profile (FRP) included having a hobby and lower BMI, with total threat avoidant vigilance score below the mean showing a highly significant adjusted association (OR=0.30, CI=0.12–0.78, p=0.01). Disturbances from other people and listening to emotionally disturbing occurrences had a significant inverse multivariate relation with FRP.

Stress-Related Disorders as Occupational Sentinel Health Events among Physicians

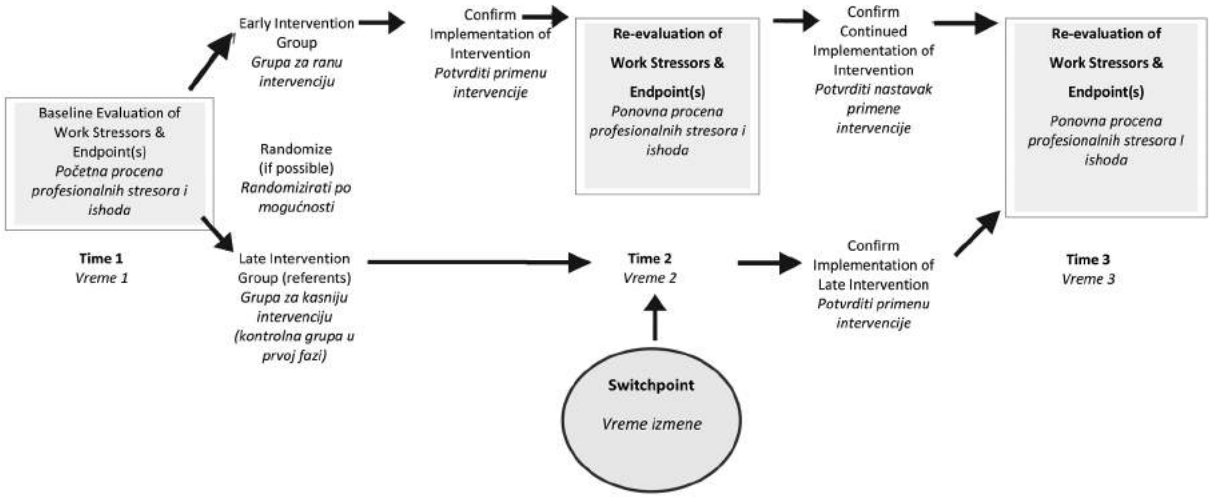
Taken together, these findings suggest that the occurrence of stress-related disorders among physicians warrants particular attention. The concept of ”occupational sentinel health events” is helpful in this context [47]. Thereby, the health problem of the individual is viewed as a potential health problem of the wider group, such that others who are also ill at the workplace are actively sought out, and the occupational hazards are identified and ameliorated [48, 49]. With regard to physicians, important warning signs would be that at a given workplace there has been a physician suicide occurrence or attempt, one or more physicians with severe burnout, or a physician, especially if young, who has had a myocardial infarction or other serious cardiovascular or cerebrovascular event. Insofar as the total OSI score is also very high, chances are that this is not an isolated occurrence. Rather, it is likely that dangerous conditions are present for other physicians at the same workplace. These considerations have been pivotal in spurring debate about whether, e.g., myocardial infarction among physicians should be recognized as a work-related disease [50].

A More Proactive Role for Physicians vis-à-vis our Work Environment and for Patients

Clearly, then, a more proactive role for physicians can be envisioned with response to our own working environment, as well as that of our patients. Specifically, we are the ones called upon to decide about our patients' work fitness. Within that framework, we are obliged to make recommendations to improve the working conditions of our patients. This is especially important since work-stress related health disorders are increasingly recognized as a major public health problem, affecting millions of people, with enormous human and economic costs [51–55]. At the same time, as illustrated in the above-reviewed empirical data, we physicians face a heavy job stressor burden which is strongly implicated with adverse health outcomes. The key challenge is to identify the most effective strategies to lower the risk of work-stressor related illness, both for our patients and for ourselves. The critical gap is the lack of evidence-based guidelines.

Intervention Studies for Etiologic Research and Prevention

Randomized controlled intervention studies represent the strongest line of evidence in etiologic research. Moreover, they provide a very practical means of testing the efficacy of prevention strategies [3]. With regard to ameliorating occupational stressors, one of the most robust intervention study designs is the ”Interrupted time-series with switching replication” [56]. The intervention is first ap-



Scheme 1. Interrupted Time Series with Switching Replication [Ref. 56] Design of an Occupational Stressor Intervention Study
Shema 1. *Dizajn interventne studije o profesionalnim stresorima: „Prekidana vremenska serija sa naizmeničnom replikacijom"*

plied to one group, with another group serving as referent. At a designated switch point, the intervention is then implemented in the latter, with relevant follow-up comparisons performed from baseline forward in both groups. **Scheme 1** provides a schematic summary of this study design.

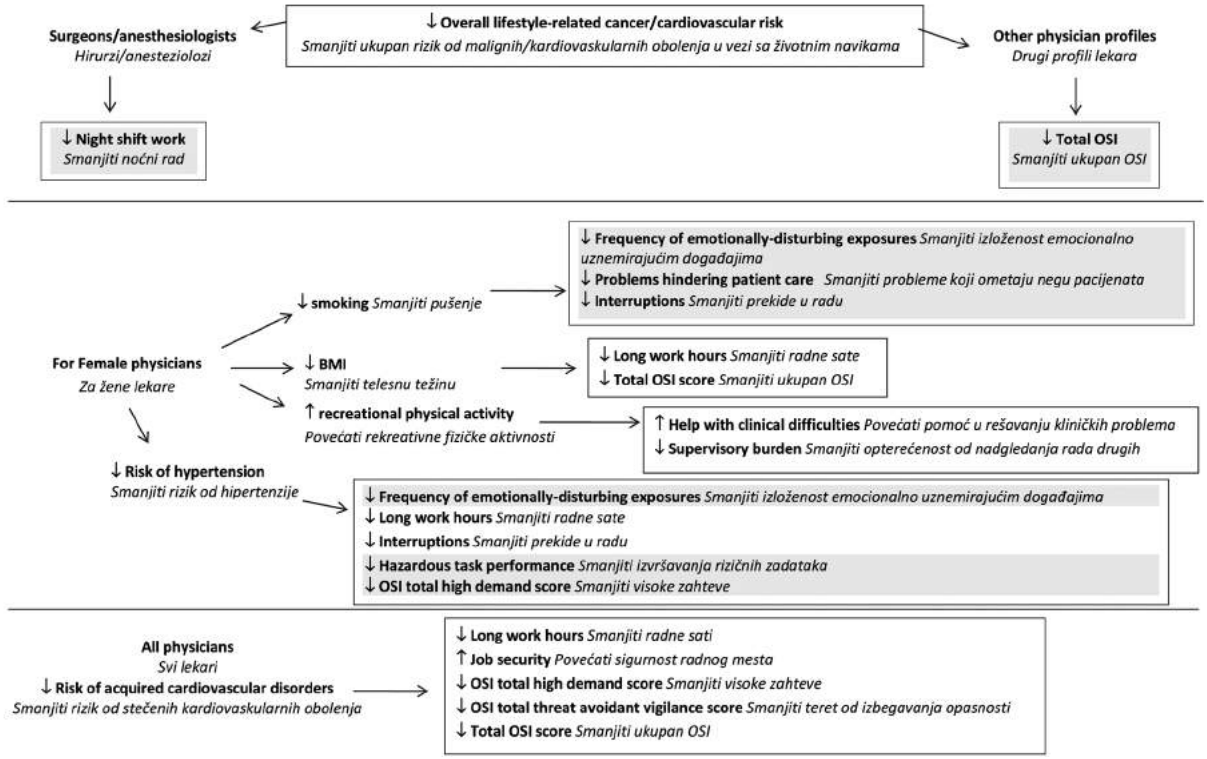
Observational research can inform decisions about which interventions might be most effective. Thus, for example, we have suggested that since among surgeons and anesthesiologists, nightshift work is identified for its adverse impact upon lifestyle-related risk of cancer and cardiovascular disease, the conditions of nightshift work should be a specific target of interventions aimed at lowering this risk among surgeons and anesthesiologists. Among physicians in other profiles, our results suggested that lowering the overall job stressor burden *per se* would be the recommended intervention strategy which should be tested [18]. How these empirical results among physicians could inform intervention strategies are summarized in **Scheme 2**. On-site visits to the worksite can yield invaluable insights about potentially modifiable stressors, as illustrated for example in Ref. [57]. Participatory action research [58] whereby the persons involved in the study actively and iteratively contribute to the intervention design represents a particularly promising strategy. This has been formulated for our profession as by "physicians for physicians" [12, 14, 59].

Our broader clinical experience in developing return-to-work (RTW) strategies for patients with stress-related disorders is also informative. We have found that lowering the overall job stressor burden as assessed through the OSI, is consistently associated with improved chances of successful RTW [11, 52]. The clinician's experience applying workplace modifications for individual patients can be invaluable in a larger framework. Namely, those changes that clearly were effective for a given patient or patient(s) could be extended into the public health realm, informing various levels of preventive workplace interventions [47], as illustrated in **Scheme 3**.

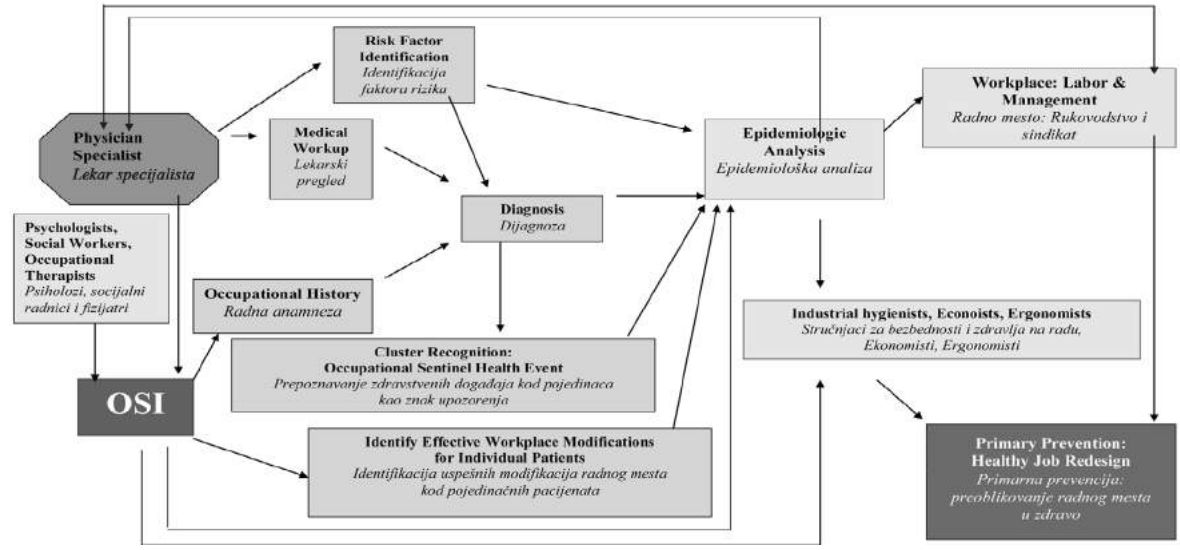
These realizations have important implications for health protection and work fitness, as has been emphasized, e.g. with regard to "occupational cardiology" [60], which was introduced several decades ago, but unfortunately, is not yet widely incorporated into clinical practice. Our efforts suggesting that myocardial infarction among physicians be considered as a potentially work-related disease [50] have been developed within this context. Most importantly, these efforts would pave the way for prevention-oriented workplace interventions aimed at lowering the risk of cardiovascular disease as well as other stress-related disorders among as well as beyond our profession.

Sub-specializations in Occupational and Stress Medicine for Physicians in Other Specialties?

It is clear that the physicians of many specialties encounter innumerable patients whose clinical state has been profoundly affected by their work conditions. However, most specialty training outside occupational medicine (with some noteworthy exceptions, especially pulmonary medicine) has generally afforded little attention to the work environment. Consequently, physician specialists generally lack the expertise needed to handle work-related issues effectively. On the other hand, occupational medicine specialists often do not have sufficient training in other medical disciplines to provide the needed care for patients with more serious health disorders. A closer integration between occupational and stress medicine training on the one hand, and other medical specialties would be an important step in this process. **Scheme 3** summarizes this dynamic, larger frame-



Scheme 2. Occupational Intervention Strategies for Physicians Informed by Observational Findings from the OSI
Shema 2. *Intervencije na uslovima rada lekara bazirane na empirijskim istraživanjima primenom OSI*



Scheme 3. The Role of the Clinician/Physician Specialist and the OSI in the Larger Framework of Creating Healthy Workplaces, as adapted from Refs. [11, 47]
Shema 3. *Uloga lekara/kliničara specijaliste i OSI u kreiranju zdravog radnog mesta, prilagođena iz Ref. [11, 47].*

work in which such clinicians could best contribute to develop and implement evidence-based guidelines for creating healthier work places for our profession as well as for our patients.

Where are We Now and where do We Go from Here?

Much has changed since the earlier days when occupational medicine was a leading discipline

with broad influence and extensive empowerment to implement recommendations in the working lives of millions of people in this region. Not only has this empowerment been severely eroded, but, as discussed, the nature of work-related factors that impact upon health has also changed profoundly, not only here, but globally. We have outlined some strategic directions based upon empirical evidence garnered in large measure from this region. Intervention studies in which job stressors are ameliorated as a therapeutic/preventive modality are urgently needed and the logical starting point is with our own profession. We have also suggested how the relevant clinical competence should be enhanced. Alongside this clinical enhancement should be the full restoration of the empowerment of these physician specialists to implement work-related recommendations. A participatory action research perspective [58] by physicians and for physicians and for our patients would be the recommended approach.

References

1. Stanković D, Beritić T, Cvetanov V, Killibarda M, Markičević A, Mikov M, et al, eds. Medicina rada. Beograd, Zagreb: Medicinska Knjiga; 1984.
2. South East Europe Workplace Academy - SEEWA 2011 Healthy workplaces for health workers - June 27 to July 2, 2011 – Zagreb: SEEWA; 2011.
3. Belkić K, Landsbergis P, Schnall P, Baker D. Is job strain a major source of cardiovascular disease risk? Scand J Work Environ Health 2004;30:85-128.
4. Hu H, Speizer F. Influence of environmental and occupational hazards on disease. In: Braunwald E, Fauci A, Kasper D, Hauser D, Longo D, Jameson J, eds. Harrison's principles of internal medicine. 15th ed. New York (NY): McGraw-Hill, Inc; 2001. p 19–21.
5. Karasek RA. Job demands, job decision latitude and mental strain: Implications for job redesign. Adm Sci Q 1979;24:285-307.
6. Karasek RA, Theorell T. The demand-control-support model and CVD. Occup Med 2000;15:78-83.
7. Siegrist J. Adverse health effects of high-effort/low-reward conditions. J Occup Health Psych 1996;1:27-41.
8. Siegrist J, Peter R. Measuring effort-reward imbalance. Düsseldorf: University of Düsseldorf; 1999.
9. Kivimäki M, Virtanen M, Elovainio M, Kouvonen A, Väänänen A, Vahtera J. Work stress in the etiology of coronary heart disease: a meta-analysis. Scand J Work Environ Health 2006;32:431-42.
10. Stansfeld S, Candy B. Psychosocial work environment and mental health: a meta-analytic review. Scand J Work Environ Health. 2006;32:443-62.
11. Belkić K, Savić Č. Job stressors and mental health: a proactive clinical perspective. London: World Scientific Publishers; 2013.
12. Belkić K, Savić Č. The occupational stress index: an approach derived from cognitive ergonomics applicable to clinical practice. Scand J Work Environ 2008;32(Suppl 6):169-75.
13. Welford AT. The measurement of sensory-motor performance: survey and reappraisal of twelve years' progress. Ergonomics 1960;3:189-230.
14. Belkić K. The occupational stress index: an approach derived from cognitive ergonomics and brain research for clinical practice. Cambridge: Cambridge International Science Publishing; 2003.
15. Gaillard AWK. Comparing the concepts of mental load and stress. Ergonomics 1993;36:991-1005.
16. Fuller R. A conceptualization of driving behaviour as threat avoidance. Ergonomics 1984;27:1139-55.
17. Belkić K, Nedić O. Workplace stressors and lifestyle-related cancer risk factors among female physicians: assessment using the occupational stress index. J Occup Health 2007;49:61-71.
18. Belkić K, Nedić O. Night work, total occupational burden and cancer/cardiovascular risk factors in physicians. Med Pregl 2012;65(11-12):461-9.
19. Belkić K, Pavlović S, Djordjević M, Uglješić M, Micković Lj. Determinants of cardiac risk in professional drivers. Kardiologija 1992;13:145-9.
20. Belkić K, Savić Č, Theorell T, Rakić Lj, Ercegovac D, Djordjević M. Mechanisms of cardiac risk among professional drivers. Scand J Work Environ Health 1994;20:73-86.
21. Djindjić N, Jovanović J, Djindjić B, Jovanović M, Jovanović J. Associations between the occupational stress index and hypertension, type 2 diabetes mellitus, and lipid disorders in middle-aged men and women. Ann Occup Hyg 2012;56:1051-62.
22. Emdad R, Belkić K, Theorell T, Cizinsky S, Savić Č, Olsson K. Work environment, neurophysiologic and psychophysiologic models among professional drivers with and without cardiovascular disease. Stress Med 1997;13:7-21.
23. Emdad R, Belkić K, Theorell T, Cizinsky S. What prevents professional drivers from following physicians' cardiologic advice? Psychother Psychosom 1998;67:226-40.
24. Nedić O, Belkić K, Filipović D, Jocić N. Work stressors among physicians with the acquired cardiovascular disorders: assessment using the occupational stress index. Med Pregl 2008;61:22-34.
25. Nedić O, Belkić K, Filipović D, Jocić N. Gender as an important effect modifier between exposure to work stressors among physicians and the occurrence of cardiovascular disease. Med Pregl 2008;61:343-9.
26. Nedić O, Belkić K, Filipović D, Jocić N. Job stressors among female physicians: relation to having a clinical diagnosis of hypertension. Int J Occup Environ Health 2010;16:330-40.
27. Uglješić M, Belkić K, Simeunovic-Micković Lj, Vukajlović M. Implementation of a plan for cardiac prevention among professional drivers as a high-risk group. Srp Arh Celok Lek 1992;120(Suppl 1):49-51.
28. McMichael A. Standardized mortality ratios and the "healthy worker effect". Scratching the surface. J Occup Med 1976;18:165-8.
29. Van Dijk F. Work-related musculoskeletal and mental disorders. Central Eur J Occup Environ Med 1995;1:292-305.
30. Aasland OG, Ekeberg O, Schweder T. Suicide rates from 1960 to 1989 in Norwegian physicians compared with other educational groups. Soc Sci Med 2001;52:259-65.
31. Hawton K, Agerbo E, Simkin S, Platt B, Mellanby R. Risk of suicide in medical and related occupational groups: a national study based on Danish case population-based registers. J Affective Disord 2011;134:320-6.
32. Schernhammer E, Colditz G. Suicide rates among physicians: a quantitative and gender assessment (meta-analysis). Am J Psychiatry 2004;161:2295-302.

33. Arigoni F, Bovier P, Sappino A. Trend of burnout among Swiss doctors. Swiss Med Wkly 2010; 140:w13070.

34. Kumar S, Hatcher S, Huggard P. Burnout in psychiatrists. Int J Psychiatry Med 2005;35:405-16.

35. Balch C, Shanafelt T. Combating stress and burnout in surgical practice. Adv Surg 2010;44:29-47.

36. Shanafelt T, Boone S, Tan L, Dyrbye L, Sotile W, Satele D, et al. Burnout and satisfaction with work-life balance among US physicians relative to the general US population. Arch Intern Med 2012;172:1377-85.

37. Frank E, Dingle A. Self-reported depression and suicide attempts among U.S. women physicians. Am J Psychiatry 1999;156:1887-94.

38. Fridner A, Belkić K, Marini M, Minucci D, Pavan L, Schenck-Gustafsson K. Recent suicidal ideation among female university hospital physicians in Sweden and Italy: cross-sectional associations with work stressors. Gender Med. 2009;6:314-28.

39. Fridner A, Belkić K, Minucci D, Pavan L, Marini M, Pingel B, et al. The work environment and recent suicidal thoughts among male university hospital physicians in Sweden and Italy. Gender Med 2011;8:269-79.

40. Eliot RS. Stress and the heart. Mt. Kisco: Futura Publishing; 1974.

41. Carpenter L, Swerdlow A, Fear N. Mortality of doctors in different specialities: findings from a cohort of 20 000 NHS hospital consultants. Occup Environ Med 1997;54:388-95.

42. Nedić O, Filipović D, Solak Z. Job stress and cardiovascular diseases among health workers. Med Pregl 2001;54:423-31.

43. Schwartz J, Pickering T, Landsbergis P. Work-related stress and blood pressure: current theoretical models and considerations from a behavioral medicine perspective. J Occup Health Psychol 1996;1:287-310.

44. Uchiyama S, Kurasawa, T, Sekizawa T, Nakatsuka H. Job strain and risk of cardiovascular events in treated hypertensive Japanese workers: hypertension follow-up group study. J Occup Health 2005;47:102-11.

45. Nedić O. Occupational stressors and physician health (dissertation). Novi Sad, University of Novi Sad; 2006.

46. Nedić O, Belkić K. Workplace health promotion among surgeons and anesthesiologists, 12th Congress of occupational medicine. Serbia, Zlatibor, October 2013. Belgrade: SLD; 2013.

47. Fisher J, Belkić K. A public health approach in clinical practice.Occup Med 2000;15:245-53.

48. Markowitz S. The role of surveillance in occupational health. In: Rom W, ed. Environmental and occupational medicine. Philadelphia: Lippincott-Raven Publishers; 1998. p. 19-29.

49. Mullan R, Murthy L. Occupational sentinel health events: an updated list for physician recognition and public health surveillance. Am J Ind Med 1991;19:775-99.

50. Nedić O. Akutni infarkt miokarda kao povreda na radu ili profesionalno oboljenje. Pravni Život 2006;1(9):497-510.

51. Ahola K, Virtanen M, Honkonen T, Isometsä E, Aromaa A, Lönnqvist J. Common mental disorders and subsequent work disability: a population-based health 2000 study. J Affect Disord 2011;134:365-72.

52. Belkić K. Return-to-work in Scandinavia: experience and insights with a focus on Sweden. In: Talmage JB, Melhorn JM, Hyman M, eds. A physician's guide to return to work. Chicago: American Medical Association Press; 2011. p. 465-72.

53. Guthrie R, Ciccarelli M, Babic A. Work-related stress in Australia: the effects of legislative interventions and the cost of treatment. Int J Law Psychiatry 2010;33:10-5.

54. Pro J. Working with common psychiatric problems. In: Talmage JB, Melhorn JM, Hyman M. Physician's guide to return to work. Chicago: American Medical Association Press; 2005. p. 305-20.

55. Sultan-Taieb H. Modeling the economic burden of diseases imputable to stress at work. Eur J Health Econom 2005; 50:16-23.

56. Beehr T, O'Hara K. Methodological designs for the evaluation of occupational stress interventions. In: Kasl S, Cooper C, eds. Research methods in stress and health psychology. New York: John Wiley; 1987. p. 79-112.

57. Belkić K, Schnall P. On a San Francisco public transport line: burden and consequences upon the human operator. The Job Stress Network Website: Center for Social Epidemiology (www.workhealth.org), 2000.

58. Israel B, Goldenhar L, Baker E, Heaney C. Occupational stress, safety and health: conceptual framework and principles for effective prevention interventions. J Occup Health Psychol 1996;1:261-86.

59. Savić Č. The physician and stress.17th Meeting in Sombor Medical Society of Vojvodina. Sombor: DLV; 2002. str. 52-4.

60. Maisano G, Gobbato F, Julian D, Mulcahy R. Workshop on occupational cardiology. Eur Heart J 1988;9(Suppl L):1-131.

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ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

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Original study
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MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF ALTERNATIONS IN THE ORAL CAVITY CAUSED BY *CANDIDA ALBICANS* – EXPERIMENTAL WORK

MORFOLOŠKA I MORFOMETRIJSKA ANALIZA PROMENA U USNOJ DUPLJI KOJE JE IZAZVALA CANDIDA ALBICANS – EKSPERIMENTALNI RAD

Ljiljana KESIĆ[1], Radojka DELIĆ[2], Dragan MIHAILOVIĆ[3], Milica S. PETROVIĆ[1] and Tijana Đ. DELIĆ[4]

Summary

Introduction. Candidiasis has become a human disease of increasing importance in the last decades. The aim of the study is to establish pathomorphological alterations caused by the blastospores of the *Candida albicans* as well as morphometric alterations. **Material and Methods.** The experiment was carried out on 2.5-month-old rats, weighting 110–130 g. The study sample was divided into the animals infected by a submucous inoculation in the periodontal region and the controls. The gingival specimens were taken, preparations were done and stained by the hematoxylin-eosin and Periodic acid Schiff methods. **Results.** The following alterations were found out by the stereological analysis: an average volume of nuclei of the gingival epithelial cells was 111.82 μm^3 (SD=25.34) on the first day. A statistically significant increase in the volume of nuclei in the experimental group began to occur from the fourth day (202.97 μm^3; SD=31.16, $p<0.05$) and the highest value of the nuclei volume was found out on the eight day of the experiment (316.83 μm^3; SD=40.15). **Conclusion.** Blastospores of *Candida albicans* are pathogenic for the gingival tissue where they cause degenerative necrotic alterations of the granulomatous character and after the fourth day from the inoculation, the development of the pseudohyphae was observed. The obtained values of stereologic measurement show the acute increase in the volume of nuclei.

Key words: Mouth; Candida albicans; Candidiasis; Rats; Gingiva; Spores, Fungal

Sažetak

Poslednjih godina kandidoza predstavlja oboljenje od posebnog značaja. Cilj ovog istraživanja bio je da se utvrde i ispitaju patomorfološke promene uzrokovane blastosporama kandide (*Candida albicans*) kao i morfometrijske promene. **Materijal i metode.** Eksperimentalna studija je urađena na pacovima starosti 2,5 meseci, težine 110−130 g. Životinje su inficirane submukoznom inokulacijom u region parodonta. Takođe, formirana je kontrolna grupa. Uzimani su isečci sa gingive, napravljeni preparati su bojeni sa hematoksilin-eozin i perjodna kiselina-*Schiff* metodom. **Rezultati.** Stereološkom analizom utvrđene su sledeće promene: u prvom danu srednja vrednost zapremine nukleusa epitelnih ćelija gingive iznosila je 111,82 μm^3 (SD = 25,34). Zapaženo je statistički značajno povećanje zapremine jedra od četvrtog dana (202,97 μm^3; SD = 31,16, $p < 0,05$), a najveća vrednost zapremine jedra iznosila je osmog dana eksperimenta (316,83 μm^3; SD = 40,15). **Zaključak.** Blastospore kandide (*Candida albicans*) patogene su za tkivo gingive, gde uzrokuju degenerativne nekrotične alteracije granulomatoznog karaktera. Posle četvrtog dana od inokulacije, pronađene su pseudohife. Dobijene vrednosti stereometrijske analize pokazale su postojanje akutnog uvećanja zapremine jedara.

Ključne reči: Usna šupljina; Candida albicans; Kandidijaza; Pacovi; Gingiva; Spore gljivica

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In memory of my father Prof. dr. Georgi Penev (1933-2012), Medical faculty University of Niš, Institute for Pathological Anatomy

Introduction

Candida species fungi are commonly present in healthy individuals, and *Candida albicans* is the most prevalent species [1, 2]. The leading cause of candidiasis, *Candida albicans*, is a dimorphic fungus that resides as a commensal of the oral mucosa and the gastrointestinal tract mucosa. Changes in the oral ecosystem or in the immunological system of the host can lead to candidiasis development [2–4]. Candidiasis has become a human disease of increas-

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Abbreviations

HIV – human immunodeficiency virus
AIDS – acquired immunodeficiency syndrome
HE – hematoxylin-eosin

ing importance in the last decades due to the increasing number of patients with immunological involvement associated with the infection by the human immunodeficiency virus (HIV) and the use of immunosuppressive agents after organ transplantation or antineoplastic therapy [1]. In immunocompromised hosts, however, saprophytic colonization often leads to opportunistic mucosal or life-threatening deep organ infection. Invasion of the human gastrointestinal mucosa by *Candida albicans* and its passage across the bowel wall into the bloodstream is an important portal of entry for this opportunistic pathogen in the neutropenic host, leading to systemic or disseminated candidiasis [5]. In addition, hematogenous candidiasis is a frequent complication in treatment of patients with acute leukemia [6]. Many researchers developed several experimental models in rats in order to understand the mechanisms related to the pathogenesis of oral candidiasis. The oral cavity of these animals is easily colonized by Candida and develops similar lesion in relation to those observed among human beings [1, 3]. Many predisposing factors for oral candidiasis have been studied in experimental models, such as: broad-spectrum antibiotics therapy [7, 8], the use of acrylic prosthesis [9], diabetes mellitus [10], topical use of corticosteroids [11], xerostomia [12, 13] and immunosuppressive therapy [4, 14]. An important cofactor associated with the pathogenesis of oral candidiasis appears to be the virulence of the infecting organism [15, 16]. The specific features of the fungus that contribute to the development of oral candidiasis include its ability to adhere to and colonize the oral mucosa [17], its ability to form cylindrical appendages termed germ tubes [18], and its cell surface hydrophobicity [19]. In addition, phenotypic and genotypic switching [20, 21], extracellular aspartyl proteinase secretion [22, 23], and phospholipase production [24] appear to play a subsidiary role in the pathogenicity.

Animal models represent powerful tools in elucidating the molecular and cellular pathogenesis of candidiasis (previously reviewed in [1, 3]). The principal advantage in studying animals instead of human beings is that the animal and its environment can be controlled [4], allowing a precise cause-and-effect longitudinal analysis of host-pathogen interactions. In addition, these models obviate the procurement of tissue samples from human patients, which can often be problematic. The usefulness of animal models of candidiasis includes not only the study of pathogenesis but also the *in vivo* assessment of novel antifungals, immunomodulators and potential Candida vaccines [25, 26]. The aim of thix study was to establish pathomorphological alterations caused by the blastospores of *Candida albicans* as well as morphometric alterations in the rats.

Material and Methods

The experiment was carried out on 2.5-month-old rats, weighting 110–130 g. A group of ten rats was infected with blastospores of *Candida albicans* in the dosage of 400.000 in 0.5 ml of physiological solution for an animal (determined in the Spenser chamber). The animals were infected by a submucous inoculation in the periodontal region. The control group consisted of three animals which were kept under the same conditions as those for the experimental group. The rats were sacrificed after 24 hours, after the second, fourth, sixth and eighth days from the moment of infection. The cuts of gingival tissue were taken, preparations were done and stained by the hematoxylin-eosin (HE) and Periodic acid Schiff (PAS) methods.

Stereologic analysis of the average volume of the nuclei of the gingival epithelial cells was also carried out according to the Gunderssen "nucleator principle" (1988).

The sinus-dependent test system was used to measure the intercept length according to the formula $Vv = (l \times \pi/3)$. The x100 objective was used.

The Student t-test was used for the statistical analysis of the obtained results.

Results

The alteration took place only at the gingival level in the gingival tissue in the acute phase up to the fourth day from the infection **(Figure 1)**.

Inflammatory-edematous alterations were ascertained by the presence of the budding blastospores and pseudomicellar fibers in the gingival tissue in the acute phase and from the fourth day onwards granulomatous alterations occurred with abscess formation in addition to numerous predominantly eosinophile elements and pseudohyphae. Giant cells of the Langhans type and the foreign body type were present in complexes. The alterations appeared due to the effects of blastospores and candidine, their metabolic product (**figures 2 and 3**).

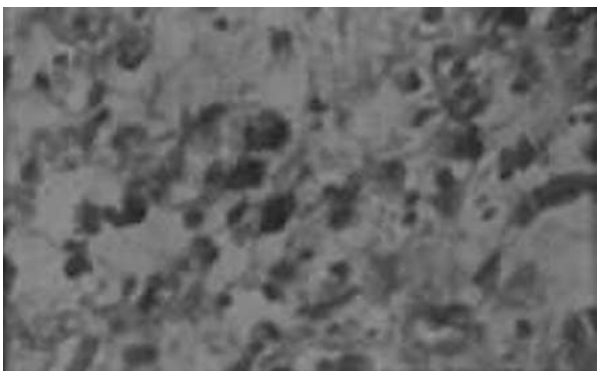

Figure 1. Gingival tissue with edema and blastospores. HE, X 400.
Slika 1*. Tkivo gingive sa edemom i blastosporama HE, X 400*

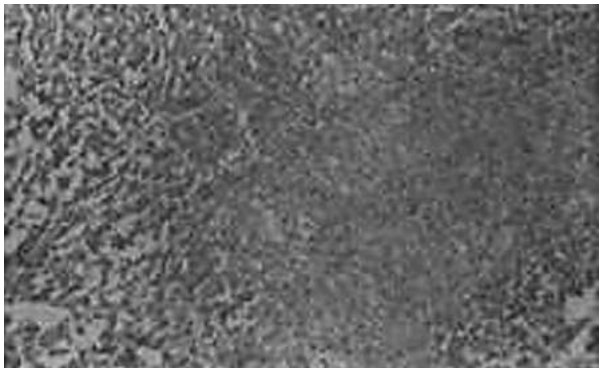

Figure 2. Edematous gingival tissue, diffuse infiltration with inflammatory elements and necrosis in the central part. HE, X 400.
Slika 2. *Edematozno tkivo gingive, difuzna infiltracija sa inflamatornim elementima i nekrozom u centralnom delu HE, X 400*

Figure 3. Subepithelial soft tissue with *Candida albicans* plaque. HE, X 400.
Slika 3. *Subepitelijalno meko tkivo sa plakom Kandide albikans HE, X 400*

The development of excess fibrous connective tissue, that is fibrosis and sclerosis, occurred in the chronic phase (**Figure 4).**

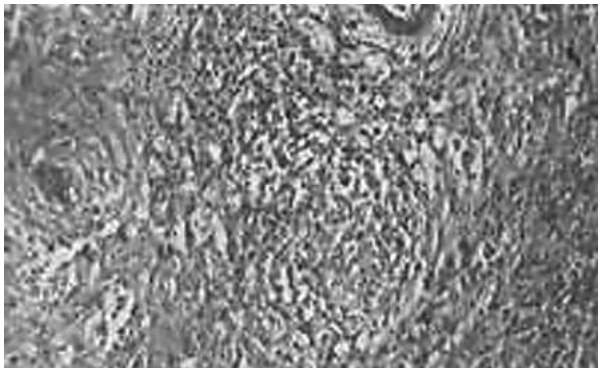

Figure 4. Gingival tissue with granulomatous process, necrotic fields and *Candida albicans* blastospores. HE, X 400.
Slika 4. *Tkivo gingive sa granulomatoznim procesom, nekrotičnim poljima i blatosporama Kandide albikans HE, X 400*

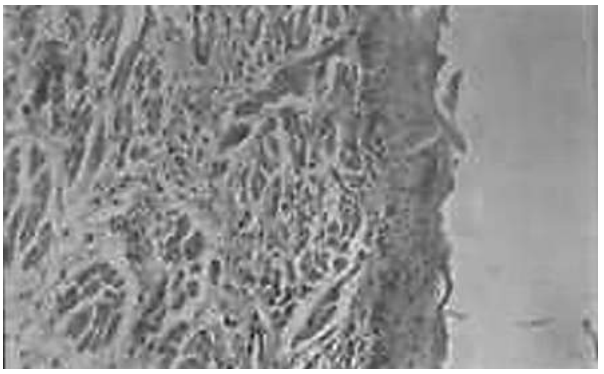

Figure 5. Control sample without pathohistologic alterations: intact epithelial lamina and subepithelial muscle tissue. HE, X 400.
Slika 5. *Kontrolni uzorak bez patohistoloških promena: neoštećena epitelijalna lamina i subepitelijalno mišićno tkivo HE, X 400*

The control sample **(Figure 5)** showed intact epithelial lamina with subepithelial muscle tissue.

The following alterations were determined by the stereological analysis:

– an average volume of nuclei of the gingival epithelial cells on the first day was 111.82 μm^3, (SD=25.34).

– a statistically significant increase in the volume of nuclei in the experimental group began to occur from the fourth day (202.97 μm^3; SD=31.16, $p<0.05$);

– the highest value of the nuclei volume was found out on the eight day of the experiment (316.83 μm^3; SD=40.15).

Discussion

The animals that inoculated by *Candida albicans* in the oral cavity developed clinical and microscopy lesions of candidiasis in the tongue dorsum even without presenting predisposing factors, such as the administration of antibiotics, immunosuppression, carbohydrate-rich diet or xerostomia. These data confirm that the experimental candidiasis can be induced by a simple inoculation of a pathogenic strain of *Candida albicans* [28]. The recognition that Candida is an important pathogen, particularly in the immunocompromised host, has resulted in a vast body of *in vitro* investigations evaluating its virulent attributes in an attempt to elucidate the pathogenesis of the disease. The progress made in understanding some of these features, such as the mechanisms that result in adherence to host tissues [29], cell surface hydrophobicity [30], switching phenomena of the yeast [22, 31], secretion of aspartyl proteinases [24], and phospholipase production [25], is very impressive. Nonetheless, *in vivo* studies either in humans or in animals are essential to elucidate and fully comprehend the mechanisms leading to candidal infection. The host oral defenses against Candida essentially fall into two categories: nonspecific immune

mechanisms (e.g., integrity of the mucosae, commensal bacteria, polymorphonuclear leukocytes, macrophages, and salivary factors) and specific immune mechanisms (e.g., serum antibodies, secretory antibodies, and cell-mediated immunity) [32]. The stratified squamous epithelium of the oral mucosa forms a continuous surface that protects the underlying tissues and functions as an impervious, mechanical barrier. The protection so provided is dependent on the degree of keratinization and the continuous desquamation or shedding of epithelial cells. Indeed, the latter mechanism is considered to play a pivotal role in maintaining a healthy oral mucosa and in limiting candidal colonization and infection. The interaction between *Candida species* and the commensal microbial flora is perhaps the next critical mechanism modulating oral candidal colonization [33]. The commensal flora regulates yeast numbers by inhibiting the adherence of yeasts to oral surfaces by competing for sites of adherence as well as for the available nutrients. A number of studies have also shown, both *in vivo* in gnotobiotic mice and *in vitro,* that candidal colonization of epithelia could be suppressed by streptococci, which are the predominant resident commensals of oral mucosal surfaces [33–35]. Consequently, the process of infection can be viewed as a competition between the ability of fungal cells to multiply and the host antimicrobial response. Obviously, for an infected host to survive and recover, it is crucial to impede the ability of pathogens to multiply [36]. Although different species of Candida, such as *Candida glabrata, Candida tropicalis, Candida krusei* and *Candida dubliniensis,* are at present recognized as increasing opportunistic pathogens specially in HIV infected individuals and acquired immunodeficiency syndrome (AIDS) patients, *Candida albicans* still remains the most common yeast isolated in humans [37]. In some ways, it is surprising that *Candida albicans* is uniquely associated with animals and human, as it has no specific nutrient requirements that would prevent it from surviving in the outside environment [38]. The use of mouse models appears appropriate since progression of both systemic and oral candidiasis closely resembles that observed in humans [39].

Conclusion

Based on the results obtained from the experimental testing of effects of blastospores of *Candida albicans* the following conclusions can be drawn:

1. Blastospores of *Candida albicans* given in dosage of 400.000 in 0.5 ml of the physiological solution are pathogenic for the gingival tissue where they cause degenerative necrotic alterations of the granulomatous character. We suppose that candidine, as a metabolic product of *Candida albicans*, play a great role in the pathohistological alterations of the gingiva.
2. Development of the pseudohyphae was found after the fourth day from the inoculation
3. The obtained values of stereologic measurement in the acute increase of the nuclei volume is probably a consequence of the nearby focus of infection or toxic effects of *Candida albicans.*

References

1. Samaranayake YU, Samaranayake LP. Experimental oral candidiasis in animal models. Clin Microbiol Rev 2001;14:398-429.

2. Webb BC, Thomas CJ, Wilcox MD, Harty DW, Knox KW. Candida-associated denture stomatitis. Aetiology and management: a review. Aust Dent J 1998;43:45-50.

3. Alenn CM. Animal models of oral candidiasis: a review. Oral Surg Oral Med Oral Pathol 1994;78:216-21.

4. de Repentigny L. Animal models in the analysis of Candida host-pathogen interactions. Curr Opin Microbiol 2004;7:324-9.

5. Walsh TJ, Merz WG. Pathologic features in the human alimentary tract associated with invasiveness of Candida tropicalis. Am J Clin Pathol 1986;85:498-502.

6. Abi-Said D, et al. The epidemiology of hematogenous candidiasis caused by different Candida species. Clin Infect Dis 1997;24:1122-8.

7. Allen CM, Beck FM, Lurie FA, Pinsky HM. Role of tetracycline in pathogenesis of chronic can didiasis of rat tongues. Infect Immun 1985;47:480-3.

8. Fisker AV, Rindon-Schiott C, Philipsen HP. Short-term oral candidiasis in rats, with special reference to the site of infection. Acta Pathol Microbiol Immunol Scand 1982;90:49-57.

9. Shakir BS, Smith CJ, Martin MV. Epithelial mitotic activity during the induction of palatal candidiasis in the Wistar rat. J Oral Pathol 1986;15:375-80.

10. Wasan KM, Conklin JS. Evaluation of renal toxicity and antifungal activity of free and liposomal amphotericin B following a single intravenous dose to diabetic rats with systemic candidiasis. Antimicrob Agents Chemother 1996;40(8):1806-10.

11. Deslauries N, Coulombe C, Carre B, Goulet JP. Topical application of a corticosteroid destabilizes the host-parasite relationship in an experimental model of the oral carrier state of Candida albicans. FEMS Immunol Med Microbiol 1995;11:45-55.

12. Jorge AOC, Totti MAG, Almeida OP, Scully C. Oral candidiasis established in the sialoadenectomized rat. J Oral Pathol Med 1993;22:54-6.

13. Jorge AOC, Totti MAG, Almeida OP, Scully C. Effect of sialoadenectomy on the carriage of Candida albicans in the mouths of rats. J Oral Pathol Med 1993;22:138-40.

14. Takakura N, et al. A novel murine model of oral candidiasis with local symptoms characteristic of oral thrush. Microbiol Immunol 2003;47:321-6.

15. McCullough MJ, Ross BC, Reade PC. Candida albicans: a review of its history, taxanomy, epidemiology, virulence attributes and methods of strain differentiation. Int J Oral Maxillofac Surg 1996;25:36-144.

16. Ruechel R. Virulence factors of Candida species. In: Samaranayake LP, MacFarlane TW, eds. Oral Candidiasis. London: Wright; 1990.

17. Kennedy MJ. Adhesion and association mechanisms of Candida albicans. Curr Top Med. Mycol 1998;2:73-169.

18. Casanova M, Cervera AM, Gozalbo D, Martinez, JP. Hemin induces germ tube formation in Candida albicans. Infect Immun 1997;65:4360-4.

19. Hazen KC. Participation of yeast cell surface hydrophobicity in adherence of Candida albicans to human epithelial cells. Infect Immun 1989;57:1894-900.

20. Scherer S, Magee PT. Genetics of Candida albicans. Microbiol Rev 1990;54:226-41.

21. Soll DR. High frequency switching in Candida albicans. Clin Microbiol Rev 1992;5:183-203.

22. De Bernardis FP, et al. Elevated aspartic proteinase secretion and experimental pathogenicity of Candida albicans isolates from oral cavities of subjects infected with human immunodeficiency virus. Infect Immun 1996;64:466-71.

23. Wu T, Samaranayake LP, Cao BY, Wang J. In vitro proteinase production by oral Candida albicans isolates from individuals with and without HIV infection and its attenuation by antimycotic agents. J Med Microbiol 1996;44:311-6.

24. Lane T, Garcia JR. Phospholipase production in morphological variants of Candida albicans. Mycose 1991;34:217-20.

25. Bromuro C, et al. Interplay between protective and inhibitory antibodies dictates the outcome of experimentally disseminated candidiasis in recipients of a Candida albicans vaccine. Infect Immun 2002;70:5462-70.

26. Roilides E, Walsh T. Recombinant cytokines in augmentation and immunomodulation of host defenses against Candida spp. Med Mycol 2004;42:1-13.

27. Allen CM, Paulson R, Duncan R. Clinical, histologic and scanning electron microscopic study of the development of chronic candidiasis of the rat tongue. J Oral Pathol Med 1989;18:352-9.

28. Kennedy MJ, et al. Molecular basis of Candida albicans adhesion. Med Mycol 1992;30:95-122.

29. Hazen KC, Brawner DL, Riesselman MH, Cutler JE, Jutila MA. Differential adherence of hydrophobic and hydrophilic Candida albicans yeast cells to mouse tissues. Infect Immun 1991;59:907-12.

30. Soll DR, Langtimm CJ, McDowell J, Hicks J, Galask R. High-frequency switching in Candida strains isolated from vaginitis patients. J Clin Microbiol 1987;25:1611-22.

31. Challacombe SJ. Immunology of oral candidiasis. In: Samaranayake LP, MacFarlane TW, eds. Oral candidiasis. London: Wright; 1990. p. 104-23.

32. Samaranayake LP, MacFarlane TW, editors. Oral candidiasis. London: Wright; 1990.

33. Liljemark WF, Gibbons RJ. Suppresion of C. albicans by human oral streptococci in gnotobiotic mice. Infect Immun 1973;8:846-9.

34. Nair R, Samaranayake LP. The effect of oral commensal bacteria on candidal adhesion to human buccal epithelial cells. J Med Microbiol 1996;45:179-85.

35. Samaranayake LP, Hamilton D, MacFarlane TW. The effect of indigenous bacterial populations on buccal epithelial cells on subsequent microbial adhesion in vitro. Oral Microbiol Immunol 1994;9:236-40.

36. Mims CA, Nash A, Stephen J. In MIMS' Pathogenesis of infectious disease. London: Academic press; 2001. p. 67-82.

37. Giammanco GM, et al. Value of morphotyping for the characterization of Candida albicans clinical isolates. Mem Inst Oswaldo Cruz 2005;5:483-90.

38. Hube B. From commensal to pathogen: stage- and tissue-specific gene expression of Candida albicans. Curr Opin Microbiol 2004;7:336-41.

39. Rozell B, Ljungdahl PO, Martínez P. Host-pathogen interactions and the pathological consequences of acute systemic Candida albicans infections in mice. Curr Drug Targets 2006;7:483-94.

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COMPARATIVE ANALYSIS OF SUCCESS OF PSORIASIS TREATMENT WITH STANDARD THERAPEUTIC MODALITIES AND BALNEOTHERAPY

KOMPARATIVNA ANALIZA USPEHA LEČENJA PSORIJAZE STANDARDNIM TERAPIJSKIM MODALITETIMA I BALNEOTERAPIJOM

Đuka NINKOVIĆ BAROŠ[1], Vesna S. GAJANIN[2], Radoslav B. GAJANIN[1] and Bogdan ZRNIĆ[2]

Summary

Introduction. Psoriasis is a chronic, inflammatory, immune-mediated skin disease. In addition to standard therapeutic modalities (antibiotics, cytostatics, phototherapy, photochemotherapy and retinoids), nonstandard methods can be used in the treatment of psoriasis. This includes balneotherapy which is most commonly used in combination with therapeutic resources. The aim of this research was to determine the length of remission of psoriasis in patients treated with standard therapeutic modalities, balneotherapy, and combined treatment (standard therapeutic modalities and balneotherapy). **Material and Methods.** The study analyzed 60 adult patients, of both sexes, with different clinical forms of psoriasis, who were divided into three groups according to the applied therapeutic modalities: the first group (treated with standard therapeutic modalities), the second group (treated with balneotherapy) and the third group (treated with combined therapy-standard methods therapy and balneotherapy). The Psoriasis Area and Severity Index was determined in first, third and sixth week of treatment for all patients. The following laboratory analysis were performed and monitored: C reactive protein, iron with total iron binding capacity, unsaturated iron binding capacity and ferritin, uric acid, rheumatoid factors and antibodies to streptolysin O in the first and sixth week of treatment. **Results.** The average length of remission in patients treated with standard therapeutic modalities and in those treated with balneotherapy was 1.77 ± 0.951 months and 1.79 ± 0.918 months, respectively. There was a statistically significant difference in the duration of remission between the patients treated with combination therapy and patients treated with standard therapeutic modalities (p=0.019) and balneotherapy (p=0.032). **Conclusion.** The best results have been achieved when the combination therapy was administered.

Key words: Psoriasis; Drug Therapy; Balneology; Combined Modality Therapy; Remission Induction; Body Surface Area; Severity of Illness Index; Diagnostic Tests, Routine

Sažetak

Uvod. Psorijaza je hronična, inflamatorna, imunoposredovana kožna bolest. Pored standardnih terapijskih modaliteta (antibiotici, citostatici, fototerapija, fotohemoterapija i retinoidi), u terapiji se primenjuju i nestandardne terapijske metode kao balneoterapija, ali najčešće kombinacija više terapijskih sredstava. Cilj rada bio je da se utvrdi dužina remisije psorijaze kod pacijenata lečenih standardnim terapijskim modalitetima, balneoterapijom i kombinovanim lečenjem (standardnim terapijskim modalitetima i balneoterapijom). **Materijal i metode.** Analizirano je 60 odraslih pacijenata oba pola, obolelih od različitih kliničkih oblika psorijaze, podeljenih u tri grupe prema primenjenim terapijskim modalitetima: grupa I (lečena standardnim terapijskim modalitetima), grupa II (lečena balneoterapijom) i grupa III (lečena kombinovanim terapijskim modalitetima − standardnim metodama i balneoterapijom). Svim pacijentima smo određivali indeks procene težine psorijaze u 1, 3. i 6. nedelji lečenja. Pratili smo laboratorijske analize: C-reaktivni protein, gvožđe, ukupni kapacitet vezanog gvožđa, kapacitet vezivanja nezasićenog gvožđa, feritin, mokraćnu kiselinu, reumatoidne faktore i antitela na streptolizin O u 1. i 6. nedelji lečenja. **Rezultati.** Prosečna dužina remisije kod pacijenata lečenih standardnim terapijskim modalitetima iznosi 1,77 ± 0,951 meseci, a kod pacijenata lečenih balneoterapijom iznosi 1,79 ± 0,918 meseci. Kod pacijenata lečenih kombinovanom terapijom, period remisije je u proseku trajao 2,47 ± 0,743 meseci. Postoji statistički značajna razlika u dužini trajanja remisije između pacijenata lečenih kombinovanom terapijom i pacijenta lečenih standardnim terapijskim modalitetima (p = 0,019), odnosno balneoterapijom p = 0,032). **Zaključak.** Primena kombinovane terapije pokazala je najbolje rezultate u lečenju psorijaze.

Ključne reči: Psorijaza; Terapija; Balneologija; Kombinovani terapijski modaliteti; Remisija; Površina tela; Indeks procene težine bolesti; Rutinski dijagnostički testovi

Introduction

Psoriasis is a chronic, inflammatory, immune-mediated skin disease characterized by increased epidermal proliferation and differentiation, and accelerated angiogenesis with dilated blood vessels and inflammation in the skin [1]. The prevalence of psoriasis is the same in both sexes, and in all socio-economic groups in the society [2]. Epidemiologic and immunogenetic studies have indicated that predisposition for psoriasis is hereditary. Belić et al. have shown the correlation among hereditary com-

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Abbreviations

HLA – human leukocyte antigen
PASI score – Psoriasis Area and Severity Index
CRP – C-reactive protein
ASTO – antistreptolysin O *titer*
UV – ultraviolet
PUVA – psoralen + UVA
Re-PUVA – retinoids and PUVA
Rf – rheumatoid factor
TIBC – total iron binding capacity
UIBC – unsaturated iron binding capacity
LSD – least significant difference test

ponent, frequency and time of appearance of psoriasis [3]. Psoriasis is associated with a certain human leukocyte antigen (HLA), and it is believed that the immune system plays an important role in the pathogenesis of psoriasis [4]. Poljački et al. have found association between psoriasis and other dermatological diseases (lichen planus, alopecia areata and vitiligo) [5]. Psoriasis is characterized by clearly defined erythematous plaques and/or papules that are covered by silver-white scaling. According to the degree of the skin involvement, psoriasis can be localized or generalized, which may turn into erythrodermia (involvement of skin greater than 90%) [6-8]. This disease may be acute, subacute, but it is usually chronic. The plaque form of psoriasis is the most common form of psoriasis [9]. The diagnosis of psoriasis is usually set on the basis of medical history, clinical and diagnostic signs (”stearin candles”, ”last skins” and ”blood droplets”) [10]. The definitive diagnosis is confirmed by pathological examination [11]. According to the percentage of the affected skin, which is determined by Evans formula for burn, calculating the Psoriasis Area and Severity Index (PASI score), which analyzes the degree of erythema, infiltration and desquamation on the affected skin. Reduction in PASI score of 75% is considered the ”gold standard” for measuring the response to therapy (PASI-75) [12–14]. In patients with psoriasis, we can see a variety of laboratory abnormalities, such as the increased concentrations of C-reactive protein (CRP), leukocytosis with a predominance of neutrophils, increased elastase, lactoferrin and α1-antitrypsin. Neutrophils play a key role in the clinical development of psoriasis. Monitoring these markers could predict relapse of the disease. In addition, there is also anemia, thrombocytosis and hyperuricemia [15–19]. Patients with psoriasis often have negative values of rheumatoid factor. Increased value of rheumatoid factor appears among elderly patients with psoriasis and in patients with psoriasis with rheumatic comorbidity [20]. Increased levels of antibodies to streptolysin O (ASO titre) were found in the serum of patients in response to infection with hemolytic streptococcus groups A, C or G. Streptococcal infection usually leads to eruptive guttate psoriasis, and deterioration of other clinical forms of psoriasis. Therefore, it makes sense to introduce antibiotic therapy in such patients [21,22].

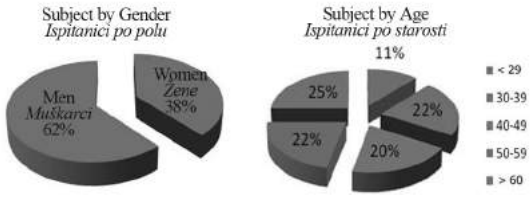
Treatment of psoriasis requires a multidisciplinary approach. The combination of multiple therapeutic agents is another approach to the treatment of psoriasis and is convenient because it reduces the cumulative toxic effects of the disease [23]. Standard therapeutic modalities include phototherapy (ultraviolet B (UVB), combined ultraviolet A (UVA) and UVB wave), photochemotherapy (UVA - with systemic photosensitizer - PUVA), combination of retinoids and PUVA (Re-PUVA), cytotoxic therapy, retinoids, antibiotic and biological therapy [24–27]. Local treatment of psoriasis includes local corticosteroid therapy, vitamin D analogues, anthralin preparations, tar and topical retinoids, calcineurin inhibitors, keratinolytics and emollients [28, 29]. In addition to standard therapeutic modality in the treatment of psoriasis, we can also use non-therapeutic procedure, such as balneotherapy [30–33]. Application of thermal mineral water containing sulfur can have a good therapeutic effect on psoriasis and other skin diseases. Thermal water also has anti-inflammatory, keratoplastic and antipruritic effect. It is used as a single modality or in combination with other therapeutic modalities [34–37].

The aim of this study was to determine the duration of remission of psoriasis in patients treated with standard therapeutic modalities, balneotherapy and combination treatment (standard therapeutic modalities and balneotherapy). It is also necessary to determine whether there is a statistically significant difference in the duration of remission of psoriasis when compared to the applied treatments of psoriasis.

Material and Methods

This prospective study included 60 adult patients, of both sexes, with different clinical forms of psoriasis. **Graph 1** shows the structure of patients by sex and age. The study sample included 60 patients, 62% men and 38% women. Most of the respondents (25.0%) were older than 60 years of age, and 11.7% younger than 29 years of age. The most common form of psoriasis in the total number of respondents was a generalized form of plaque psoriasis, which was diagnosed in 21 cases (35%). Other forms were less present **(Graph 2)**.

The patients were randomly selected from those who had consulted the doctor at the Department of Dermatology and Venereal Diseases of the Clinical Center of Banja Luka. All the respondents gave their written consent to be included in the study. Their data were entered in the questionnaire designed according to the data analyzed in the study. The questionnaire contained basic information about the patients, their diagnosis, therapeutic modality, PASI score in the first, third and sixth week, and the value of laboratory tests in the first and sixth week. Psoriasis was diagnosed according to the clinical parameters and histological analysis of the modified skin in all patients. PASI score was deter-



Graph 1. Subjects by gender and age
Grafikon 1. *Ispitanici po polu i starosti*

mined according to the severity of illness and the total area of the affected skin.

The patients were divided into three groups according to the applied therapeutic modalities:

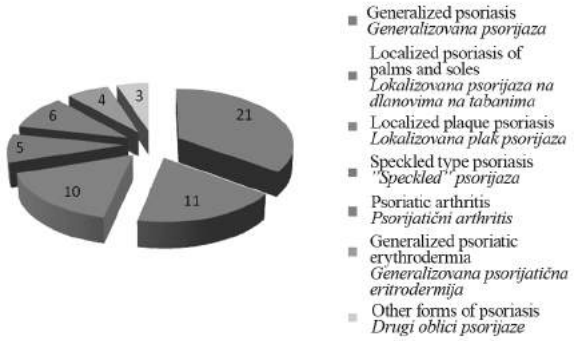
The patients at the Department of Skin and Venereal Diseases, Clinical Center of Banja Luka, treated with standard therapeutic modalities (26 patients). The standard therapeutic modality involved systemic antibiotic therapy, methotrexate, phototherapy and photochemotherapy. Local keratinolytic therapy, corticosteroid preparations, ditranol and emollients were also applied.

The patients with different clinical forms of psoriasis treated with balneotherapy at the Kulaši Spa, Republic of Srpska, for three weeks (19 patients). Water of Kulaši Spa has characteristics of healing thermal mineral water, its temperature being 30.6 degrees Celsius. Water is sterile, highly alkaline (pH 11.75) with low mineralization (168 mg/l). The spa water was used twice a day in the form of baths, hot tubs and swimming in the pool for half an hour. The patients with less severe forms of psoriasis used bath tubs, while the patients with moderate and severe forms of psoriasis swam in the pool twice a day for half an hour. After the end of treatment, the neutral local therapy (Galsanae cream) was prescribed.

The patients with psoriasis who were treated with standard therapeutic modalities combined with balneotherapy (15 patients). After hospitalization or outpatient treatment at the Department of Skin and Venereal Diseases, Clinical Center of Banja Luka, the patients were sent to the Kulaši Spa, for a period of 21 days. In cooperation with the dermatovenerologist, the spa treatment protocol was determined according to the severity of the clinical picture measured on PASI score.

Remission was followed over the period of three months. The clinical examination and PASI scores were measured in the first, third and sixth week of treatment to determine the period of remission. At the beginning and at the end of the study, all patients underwent the following laboratory tests: ASTO, Rheumatoid factor (Rf), CRP, uric acid, iron with ferritin (determined only at the end of the treatment) and total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC).

The statistical analysis of the results used descriptive and analytical statistics. For statistical



Graph 2. Distribution of patients by diagnosed form of psoriasis
Grafikon 2. *Distribucija pacijenata prema dijagnostikovanom obliku psorijaze*

analysis of data, the Statistical Package for the Social Sciences (SPSS) (version 17) was used.

Results

The average values of PASI score were determined in the first, third and sixth week of the treatment (**Table 1**). Fisher's Exact Test showed a statistically significant difference (p=0.049) in the mean valu- es of PASI score in the first week of treatment among different groups. In the first week, the patients treated with standard therapeutic modalities had PASI score values significantly higher than in the group of patients treated with balneotherapy, but in comparison with the group of patients treated with combined therapy, there were no statistically significant differences in the values of PASI scores. During the second measurement (the third week), the mean PASI scores were the highest in the group of patients treated with standard therapeutic modalities (11.05±9.550).

The factor analysis showed no statistically significant difference between the values of PASI scores among the tested groups of patients (p = 0.145). The third measurement (in the sixth week of treatment) showed that the group of patients treated with balneotherapy had the lowest mean values of PASI score (2.85 ± 2.556); whereas the patients treated with standard therapy had the highest value (5.68 ± 4.423).

There was no statistically significant difference in PASI scores between different groups of all patients (p = 0.54). The use of Robust test showed a statistically significant difference in the values of PASI score (in sixth week) between different groups of patients (p = 0.037). In order to determine which groups of patients differed significantly among themselves, Post-hoc tests (Tukey post-hoc test) were used. These statistical tests showed that there were statistically significant differences in the values of PASI scores measured in the sixth week of treatment among the patients treated with standard therapeutic modalities and balneotherapy (p = 0.043). However,

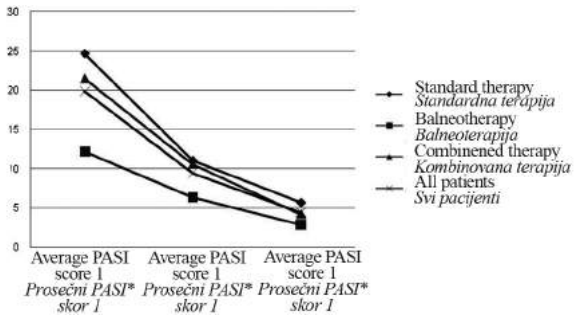
Table 1. Average PASI score determined in the first, third and sixth week of treatment with different therapeutic methods
Tabela 1. *Prosečni PASI skor određen u prvoj, trećoj i šestoj nedelji lečenja različitim terapeutskim metodama*

Average PASI score±SD *PASI Prosečni skor*	Standard therapy *Standardna terapija*	Balneotherapy *Balneoterapija*	Combination therapy *Kombinovana terapija*
First week/*Prva nedelja*	24.65±20.452	12.16±11.070	21.48±15.091
Third week/*Treća nedeljna*	11.05±9.550	6.36±6.410	10.60±7.528
Sixth week/*Šesta nedelja*	5.68±4.423	2.85±2.556	4.17±3.950
N (number of patient)/*N (broj pacijenata)*	26	19	15

PASI - indeks proširenosti i težine psorijaze

there was no difference between the group treated with combination therapy and the one treated with standard therapy (p = 0.443), and the group treated with combination therapy and balneotherapy (p = 0.577). The results of Friedman's test showed that there were statistically significant differences between the values of PASI scores determined in the first, third and sixth week of treatment in all patients (60 patients) (p = 0.000). The values of PASI score in the sixth week of measurements were statistically significantly lower than the values of PASI score in the first week (Wilcoxon's test: Z = -6.031, p = 0.000) and the values of PASI score in the third week (Wilcoxon's test: Z = - 5.626, p = 0.000). Also, the values of PASI scores determined in the third week of treatment were statistically significantly lower than the values in the first week (Wilcoxon's test: Z = - 6.031 p = 0.000). The value of PASI score during the study period of six weeks in all treated patients statistically significantly decreased because of applied therapeutic modalities **(Graph 3)**.

The average values of CRP are shown in **Table 2**. The difference between the average value of CRP at the beginning and at the end of treatment was not statistically significant in any of the groups, but after the end of treatment, there was a decrease in the value of CRP as a result of therapy. The factor analysis of the average value of CRP in the first and second measurements showed that there was no statistically



Graph 3. PASI score determined in the first, third and sixth week of treatment with different therapeutic methods
Grafikon 3. *PASI skor određen u prvoj, trećoj i šestoj nedelji lečenja različitim terapeutskim metodama*
PASI – indeks proširenosti i težine psorijaze

significant difference in CRP levels among the study groups (p = 0.326 and p = 0.605).

The therapy applied for six weeks led to an increase in the value of iron in most patients, no matter whether the patient had had a reduced iron value or the values of iron had been within the reference values. The average value of iron in all patients in the first week of treatment was 18.95±7,834 μmol/L and in the sixth week 19.10±5.996 μmol/L. The recorded increase in the value of iron in the sixth week by 0.15 μmol/L was not statistically significant (**Table 2**). The factor analysis of the average values of TIBC in relation to therapeutic modality showed that there was no statistically significant difference in both measurements among all tested patients treated with different modalities (TIBC in the first week of p = 0.062, and in the sixth week of p = 0.352). The factor analysis of the average values of UIBC in relation to therapeutic modality showed that there was no statistically significant difference in both measurements among all tested patients treated with different therapeutic modalities (UIBC in the first week of p = 0.659, UIBC in the sixth week of p = 0.698).

The average value of ferritin in all patients was 80.56 ng/mL. The highest value was in the patients treated with balneotherapy (90.17 ng/mL), and the lowest in the patients treated with standard therapeutic modalities (71.91 ng/mL). Differences in ferritin values were not statistically significant (p = 0.829).

Uric acid levels in the psoriasis patients treated with different therapeutic modalities are shown in **Table 2**. The factor analysis did not establish that these differences of mean values uric acid were statistically significant in the first and sixth week among different groups of patients (p = 0.929 and p = 0.599).

The ASTO values were tested by Kruskal-Wallis test, and the results showed that there were no statistically significant differences in the values of ASTO titer in the first week (H = 3.082, p = 0.214) and at the end of the sixth week (H = 2.525, p = 0.283) among different groups of patients.

Rf-values of the test latex and Waaler-Rose test were determined in patients during the first and at the end of the sixth week in the patients treated with different therapeutic modalities. In our study there were only few respondents with positive rheumatoid factor value.

Table 2. Average laboratory parameters by different therapeutic methods
Tabela 2. *Prosečni laboratorijski parametric po različitim terapeutskim metodama*

Laboratory parameters ±SD *Laboratorijski parametri±SD*	Standard therapy *Standardna terapija*	Balneotherapy *Balneoterapija*	Combination therapy *Kombinovana terapija*
C-reactive protein (mg/L) first week/*prva nedelja*	6,19±8,498	2,86±3,190	7,30±14,204
C-reactive protein (mg/L) sixth week/*šesta nedelja*	4,50±8,509	2,33±3,243	3,57±7,975
Iron/*gvožđe* (μmol/L) first week/*prva nedelja*	17,90±8,021	20,76±2,019	18,45±6,120
Iron/*gvožđe* (μmol/L) sixth week/*šesta nedelja*	18,56±5,292	19,70±1,706	19,29±5,421
Uric acid/*mokraćna kiselina* (μmol/L) first week/*prva nedelja*	339,61±97,647	344,26±75,990	332,07±26,210
Uric acid/*mokraćna kiselina* (μmol/L) sixth week/*šesta nedelja*	317,00±96,324	324,58±70,049	294,44±24,491

The success rate of psoriasis treatment is defined by the duration of remission. The analysis of the obtained results showed that the remission lasted for 1.77 ± 0.951 months on average in the group of patients treated with standard therapeutic modalities. In the patients treated with balneotherapy the remission lasted for 1.79 ± 0.918 months on average, whereas in the patients treated with combination therapy of remission it lasted for 2.47 ± 0.743 months on average.

Discussion

The prevalence of psoriasis is the same in both sexes and in all socio-economic groups in society [2]. There were 62% of men and 38% of women in our study sample. The average age of the participants was 48.6 (the youngest respondent was 20 and the oldest 83 years of age). The highest number of patients had stable, localized or generalized form of plaque psoriasis (51.7%), which is consistent with the literature data [9].

The PASI score is determined at the beginning, during and at the end of the treatment in order to evaluate the success of a particular treatment in psoriasis [13]. The greatest values in the PASI score in the first week were found in the group of patients treated with standard therapy (24.65 ± 20.452) and the smallest group of patients treated with balneotherapy (12.16 ± 11.070). During the measuring of PASI score, the values in the sixth week of treatment showed that the patients treated with balneotherapy had the lowest average value of 2.85 ± 2.85, and the patients treated with standard therapy had the highest value (5.68 ± 4.423). The use of Robust test showed a statistically significant difference in the values of PASI score in the sixth week among different groups of patients ($p = 0.037$). The comparative analyzes and the Tukey and least significant difference (LSD) tests showed that a statistically significant difference occured in the values of PASI score in the group of patients treated with standard therapy and balneotherapy ($p = 0.043$ and $p = 0.017$). The differences between the combination therapy and the standard therapy were not statistically significant, but the values of both groups were significantly lower than in the baseline PASI score ($p = 0.443$ and $p = 0.226$). In addition, the difference between the combination therapy and balneotherapy was not statistically significant, but the values of PASI score in the combined therapy were significantly lower compared to the PASI score values at the beginning ($p = 0.319$ and $p = 0.577$).

The results of our study indicate a positive effect of the combined treatment of psoriasis using standard therapy modalities and balneotherapy. The study on the treatment of chronic plaque psoriasis conducted in the Comano Spa in Italy in 2008 had also confirmed this result. There was a reduction of PASI score within two weeks after the implementation of combination therapy (photobalneotherapy) ($p <0.001$) [31]. Kazandijeva et al. note that climate therapy may be an alternative to conventional treatment of psoriasis [35].

CRP has been proposed as a marker for psoriasis severity assessment and monitoring of the disease because it is not based on visual assessment, unlike the PASI score [15]. The factor analysis of the average value of CRP in the first and sixth measurement showed that there was no statistically significant difference in CRP levels among the study groups ($p = 0.326$ and $p = 0.605$). However, CRP remained slightly elevated at the end of therapy. The reduction of the CRP value in all groups of patients was not statistically significant. The results are consistent with the results of Chodorowska et al. who found that the concentration of CRP remained elevated during remission, compared with healthy controls [16].

Hyperuricemia is frequently present in patients with psoriasis. This correlation was first observed by Eisen and Seegmiller in 1961. They found a correlation between concentrations of uric acid in the serum and skin changes in 30-40% patients with psoriasis [18]. The patients treated with balneotherapy had the highest average values (324.58 ± 70.049), and the patients treated with combination therapy had the lowest average values of uric acid (294.44 ± 24.491). The application of combined therapy had the best effect in reducing serum uric

acid. The average value of uric acid was 313.76 ± 87.753 in all patients. These differences in mean values of serum uric acid were not statistically significant in the first and sixth measurement in all patients ($p = 0.929$ and $p = 0.599$).

There were very few respondents in all therapeutic groups with ASTO positive values (> 200 IU/ml). ASTO values in respondents tested by Kruskal-Wallis test showed that there was no statistically significant difference in ASTO values in the first week ($H = 3.082$, $p = 0.214$) and at the end of the sixth week ($H = 2.525$, $p = 0.283$) among the different groups of patients. In order to determine the effect of different therapeutic modalities in the treatment of psoriasis, we followed our patients over a period of three months. The obtained results showed that there was no statistically significant difference in the duration of remission between the patients treated with the combination therapy and the patients treated with standard therapeutic modalities ($p = 0.019$) balneotherapy ($p = 0.032$).

It can be noted that the combination therapy showed the best results in the treatment of psoriasis. The study of synchronous application of narrowband UVB therapy and swimming in the Dead Sea in 60 ambulatory patients with psoriasis conducted by Schiffner et al. showed good results of the combination therapy in psoriasis [36]. The study of Brockow et al, done in 160 adult patients with psoriasis and PASI score exceeding 10, who were treated with balneotherapy and UVB phototherapy, showed that the combination therapy was more effective than UVB therapy at the end of sixth week [37]. Bathing in geothermal seawater combined with NB-UVB therapy in psoriasis results in faster clinical and histological improvement, produces longer remission time and allows lower NB-UVB doses than UVB therapy alone [38].

Conclusion

It has been found that there is a statistically significant difference in the duration of remission between the patients treated with the combination therapy and the patients treated with standard therapy or balneotherapy. The application of combined treatment shows the best results in the treatment of psoriasis.

References

1. Smith CH, Barker JN. Psoriasis and its management. BMJ 2006;333(7564):380-4.
2. Islam MT, Paul HK, Zakaria SM, Islam MM, Shafiquzzaman M. Epidemiologica determinants of psorisis. Mymensingh Med J 2011;20(1):9-15.
3. Belić D, Ristić-Nikolić S, Damjan S, Ratkov I, Četke M. Naslednost psorijaze. Med Pregl 2004;57(3-4):171-4.
4. Sabat R, Philipp S, Hoflich C, Kreutzer S, Wallace E, Asadullah K, et al. Immunopathogenesis of psoriasis. Exp Dermatol 2007;16(10):779-98.
5. Poljački MN, Begenešić M, Đuran VD, Matović Lj, Matić M, Jovanović M, i dr. Psorijaza i autoimuna obolenja. Med Pregl 2002;55(7-8):325-8.
6. Karadaglić Đ, izd. Dermatologija. Beograd: Vojnoizdavački zavod; 2000 (Serbian).
7. Lebwohl M. Psoriasis. Lancet 2003;361(9364):1197-204.
8. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet 2007;370(9583):263-71.
9. Meier M, Sheth PB. Clinical spectrum and severity of psoriasis. Curr Probl Dermatol 2009;38:1-20.
10. Pariser DM, Bagel J, Gelfand JM, Korman NJ, Ritchlin CT, Strober BE, et al. National psoriasis foundation clinical consensus on disease severity. Arch Dermatol 2007;143(2):239-42.
11. Weedon D, editor. David Weedon skin pathology. 2nd ed. London: Churchill Livingstone; 2002.
12. Louden BA, Pearce DJ, Lang W, Feldman SR. A simplifield psoriasis area severity index (SPASI) for rating psoriasis severity in clinick patients. Dermatol Online J 2004;10(2):7.
13. Katz KA. Psoriasis Area and Severity Index 50 as an endpoint in psoriasis trials: an uncovincing proposal. J Am Acad Dermatol 2005;53(3):547-51.
14. Carlin CS, Feldman SR, Krueger JG, Menter A, Krueger CG. A 50% reduction in the psoriasis area severity index (PASI 50) is a clinically significant endpoint in the assessment of psoriasis. J Am Acad Dermatol 2004;50(6):859-66.
15. Coimbra S, Oliveira H, Reis F, Belo L, Rocha S, Quintanilha A, et al. C-reactive protein and leucocyte activation in psoriasis vulgaris according to severity and therapy. J Eur Acad Dermatol Venereol 2010;24(7):789-96.
16. Chodorowska G, Wojnowska D, Juszkiewicz-Borowiec M. C-reactive protein and α2-macroglobulin plasma activity in medium–severe and severe psoriasis. J Eur Acad Dermatol Venereol 2004;18(2):180-3.
17. Ghosh A, Mukhopadhyay S, Kar M. Role of free reactive iron in psoriasis. Indian J Dermatol Venereol Leprol 2008;74:277-8.
18. Eisen AZ, Seegmiller JE. Uric acid metabolism in psoriasis. J Clin Invest 1961;40:1486-96.
19. Gonzales-Gay MA, Gonzales-Juanatey C, Vazquez-Rodriguez TR, Gomez-Acebo I, Miranda-Filloy JA, Paz-Carreira J, et al. Asymptomatic hyperuricemia and serum uric acid concentration correlate with subclinical atherosclerosis in psoriatic arthritis patients without clinicaly evident cardiovascular disease. Semin Arthritis Rheum 2009;39(3):157-62.
20. Sutton B, Corper A, Bonagura V, et al. The structure and origin rheumatoid factors. Immunol Today 2000;21(4):177-83.
21. Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. Br J Dermatol 2003;149(3):530-4.
22. Prinz JC. The role of streptococci in psoriasis. Hautarzt. 2009;60(2):109-15.
23. Van de Kerkof PC. Therapeutic strategies: rotational therapy and combinations. Clin Exp Dermatol 2001;26(4):356-61.
24. Lapolla W, Yentzer BA, Bagel J, Halvorson CR, Feldman SR. A review of phototherapy protocols for psoriasis treatment. J Am Acad Dermatol 2011;64(5):936-49.
25. Kalb RE, Strober B, Weinstein G, Lebwohl M. Methotrexate and psoriasis: 2009 National psoriasis foundation consensus conference. J Am Acad Dermatol 2009;60(5):824-37.

26. Colombo D, Cassano N, Altomare G, Giannetti A, Vena GA. Psoriasis relapse evalution with week-end cyclosporine a treatment: results of a randdomized, double-blind, multicentric study. Int J Immunopathol Pharmacol. 2010;23(4):1143-52.

27. Blasco AJ, Lazaro P, Ferrandiz C, Garcia-Diez A, Liso J. Efficiency of biologic agents in the treatment of moderate to severe psoriasis. Actas Dermosifiliogr 2009;100(9):792-803.

28. Halverstam CP, Lebwohl M. Nonstandard and off-label therapies for psoriasis. Clin Dermatol 2008;26(5):546-53.

29. Feldman SR, Yentzer BA. Topical clobetasol propionate in the treatment of psoriasis: a review of newer formulations. Am J Clin Dermatol 2009;10(6):397-9.

30. Nasermoaddeli A, Kagamimori S. Balneotherapy in Medicine: A Review. Environ Health Prev Med 2005;10(4):171-9.

31. Peroni A, Gisondi P, Zanoni M, Girolomoni G. Balneotherapy for chronic plaque psoriasis at Comano spa in Trentino, Italy. Dermatol Ther. 2008;21(Suppl 1):S31-8.

32. Falagas ME, Zarkadoulia E, Rafailidis P.The therapeutic effect of balneotherapy: Evaluation of the evidence from randomized controlled trials. Int J Clin Pract 2009;63(7):1068-84.

33. Roos S, Hammes S, Ockenfels HM. Psoriasis. Natural versus artificial balneotherapy. Hautarzt 2010;61(8):683-90.

34. Leslie KS, Millington GW, Levell NJ. Sulphur and skin. J Cosmet Dermatol 2004;3(2):94-8.

35. Kazandijeva J, Grozdev I, Darlenski R, Tzankov N. Climatotherapy of psoriasis. Clin Dermatol 2008;26(5):477-85.

36. Schiffner R, Schiffner RJ, Wolfl G, Landthaler M, Glassl A, Walther T, et al. Evaluation of a multicentre study of synchronous application of narrowband ultraviolet B phototherapy (TL-01) and bathing in Dead Sea salt solution for psoriasis vulgaris. Br J Dermatol 2000;142(4):740-7.

37. Brockow T, Schiener R, Franke A, Resch KL, Peter RU. A pragmatic randomized controlled trial on the effectiveness of highly concentrated saline spa water baths followed by UVB compared to UVB only in moderate to severe psoriasis. J Altern Complement Med 2007;13(7):725-32.

38. Eysteinsdóttir JH, Ólafsson JH, Agnarsson BA, Lúðvíksson BR, Sigurgeirsson B. Psoriasis treatment: faster and long-standing results after bathing in geothermal seawater. A randomized trial of three UVB phototherapy regimens. Photodermatol Photoimmunol Photomed 2014;30(1):25-34.

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REVIEW ARTICLES
PREGLEDNI ČLANCI

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TREATMENT OF NEUROSARCOIDOSIS - INNOVATIONS AND CHALLENGES

TERAPIJA NEUROSARKOIDOZE – NOVINE I IZAZOVI

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Summary
Introduction: Sarcoidosis affects the central nervous system more frequently than it used to be believed. While the cranial nerves are most frequently affected, neurosarcoidosis can involve other nervous system tissues as well. **Treatment of Neurosarcoidosis**: Although a lot of drugs have proved useful in treating neurosarcoidosis, corticosteroids are still the gold standard in treatment of these patients. Therapeutic protocols differ regarding the dose of these drugs. Symptomatic neurosarcoidosis should always be treated with pulse corticosteroid therapy. People with diabetes, high blood pressure, osteoporosis and tuberculosis should be carefully monitored, as they are prone to complications associated with treatment with corticosteroids. In cases when treatment with corticosteroids does not show the desired results or therapy is discontinued due to the development of side effects, there are other pharmacologic options, such as methotrexate, mycophenolate mofetil, cyclophosphamide, chloroquine, azathioprine, thalidomide, and infliximab. It should be noted that the treatment response to the above mentioned regimens, except for infliximab, is relatively slow compared to corticosteroids; therefore, corticosteroids should be taken into account in all states and particularly in the acute phase of the disease. **Conclusion**: It is the existence of different forms of the disease, lack of local diagnostic criteria and different and non standardized therapy that makes the treatment of this disease difficult. Despite advances in pharmacotherapy and radiological diagnosis, it is necessary to develop better diagnostic strategies in order to set the optimal therapeutic approach.
Key words: Sarcoidosis; Drug Therapy; Central Nervous System Diseases; Diagnosis; Immunosuppressive Agents; Glucocorticoids + therapeutic use

Sažetak
Uvod. Sarkoidoza zahvata centralni nervi sistem češće nego što se ranije smatralo. Dok su kranijalni nervi najčešće pogođeni, neurosarkoidoza može zahvatiti i druga tkiva nervnog sistema. **Terapija neurosarkoidoze**. Iako se dosta lekova pokazalo korisnim u lečenju neurosarkoidoze, kortikosteroidi i dalje predstavljaju zlatni standard u lečenju ovih bolesnika. Terapijski režimi se razlikuju u pogledu doziranja lekova. Simptomatska neurosarkoidoza uvek se leči pulsnim dozama kortikosteroidne terapije. Osobe sa šećernom bolesti, povišenim krvnim pritiskom, tuberkulozom i osteoporozom treba pažljivo pratiti, pošto su sklone razvoju komplikacija u vezi sa terapijom kortikosteroidima. U slučajevima kada tretman kortikosteroidima ne pokazuje željene rezultate ili je terapija prekinuta zbog razvoja neželjenih efekata, postoje i druge farmakološke opcije, poput metotreksata, mikofenolat-mofetila, ciklofosfamida, hlorokina, azatioprina, talidomida i infliksimaba. Treba napomenuti da je na navedene terapijske režime, izuzev infliksimaba, terapijski odgovor relativno spor u odnosu na kortikosteroide – dakle kortikosteroidi treba da se uzmu o obzir u svim stanjima, naročito u akutnoj fazi bolest. **Zaključak**. Upravo postojanje različitih oblika ovog oboljenja, odsustvo dijagnostičkih kriterijuma i različita i nestandardizovana terapija čine lečenje ove bolesti težim. Uprkos napredovanjima u farmakoterapiji i radiološkoj dijagnostici, potrebno je razviti bolje dijagnostičke strategije kako bi se postavio što optimalniji terapijski pristup.
Ključne reči: Sarkoidoza; Terapija; Oboljenja centralnog nervnog sistema; Dijagnoza; Imunosupresivna terapija; Glukokortikoidi + terapija

Introduction

Sarcoidosis is a systemic granulomatous disease most frequently affecting the lungs and hilar lymph nodes. The lungs are affected in 90-95% of cases, while peripheral lymph nodes are affected

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Abbreviations

CNS	– central nervous system
MR	– magnetic resonance
PET	– positron emission tomography
MTH	– methotrexate
MMF	– mycophenolate mofetil
TNF	– tumor necrosis factor
RT	– radiotherapy

in 50-70% of cases. Nowadays it is known that sarcoidosis can affect any organ. Although the involvement of the nervous system is rather rare, it can lead to serious complications.

According to the literature data, clinically manifested neurosarcoidosis is presented in about 5-15% of patients with systemic sarcoidosis. However, it is difficult to state the exact number because of a large number of subclinical cases, i.e. cases without clearly evident symptoms. Autopsy studies have shown that only half neurosarcoidosis cases are diagnosed during lifetime. Sarcoidosis can affect any part of the nervous system, most frequently displaying symptoms by cranial nerves. Chronic form of neurosarcoidosis is particularly resistant to the applied medicament therapy [1,2,3]. Corticosteroid drugs are the first line therapy, but due to their side-effects and sometimes inadequate therapeutic response, other immunosuppresive drugs have frequently been used lately. This article has been aimed at explaining the choice of drugs, their efficiency and administration at our department.

Neurosarcoidosis Treatment

Both neurosarcoidosis and sarcoidosis are diagnosed according to the clinical and radiological findings (magnetic resonance of endocranium with contrast), laboratory findings (angiotensin-converting enzyme-ACE in liquor), histopathological confirmation, provided that all other possible causes of granulomatous inflammation have been previously excluded. The most reliable diagnostic procedure is certainly the nervous tissue biopsy, showing the presence of non caseating granuloma, although it is rarely applied in clinical practice. Basically there are three clinical forms of neurosarcoidosis where appropriate therapy should be concerned:

1) Patients with neurosarcoidosis confirmed by biopsy should be treated immediately in order to avoid permanent damage of the central nervous system (CNS).

2) Initiation of therapy must be taken into consideration when it is impossible to perform biopsy but the patients have magnetic resonance (MR) and positron emission tomography (PET) scan findings indicating neurosarcoidosis and confirmed systemic sarcoidosis, and particularly in cases when infection, neoplastic and other disorders can be excluded. In these cases the therapy can be 'diagnostic' as well if there is no remission of symptoms under the applied therapy, thus suggesting that neurosarcoidosis is rather improbable.

Immunosuppresive drugs such as corticosteroids can give temporary improvement even though it is not sarcoidosis but tuberculosis or lymphoma, for example. Therapy must be administered with caution and all its risk must be taken into account.

3) An asymptomatic patient with neurosarcoidosis proved by biopsy or some other noninvasive method presents a therapy dilemma. The decision to treat these patients is individual and it is based on the localization of lesion and its evolution during time and the risk from the therapy [3, 4].

Although many drugs have been proved to be useful in neurosarcoidosis treatment, corticosteroids still present the golden standard in treatment of these patients. Therapy regimens differ regarding dosing of these drugs. Symptomatic neurosarcoidosis is always treated with pulse corticosteroid therapy. The patients with diabetes, hypertension, tuberculosis and osteoporosis should be carefully followed since they are prone to complications resulting from corticosteroid therapy. In cases when corticosteroid therapy does not give desired results or the therapy has been discontinued due to the development of side–effects, there are other pharmacological options, such as methotrexate, mycophenolate mofetil, cyclophosphamide, chloroquine, azathioprine, thalidomide and infliximab. It should be noted that the therapeutic response to the above mentioned regimens, except for infliximab, is relatively slow compared to corticosteroids; therefore, corticosteroids should be taken into account in all states and particularly in the acute phase of the disease [5].

Methotrexate (MTH), analogous to folic acid, is most frequently used in neurosarcoidosis therapy. To treat sarcoidosis, MTH can be used independently or as a therapy agent "saving", i.e. reducing the required doses of pronison in treatment of some of its clinical forms. The precise mechanism of MTH effect in sarcoidosis treatment has not been clarified so far. MTH acts as an inhibitor of growth and functions of different cell populations, as well as a specific modulator of cytokines and their production and proliferation of fibroblast. In this way methotrexate shows its anti-inflammatory effect. Lower et al. [6] found that 61% of patients treated by MTH as a replacement for corticosteroid therapy responded adequately to the treatment. When combined with corticosteroids to treat neurosarcoidosis, MTH reduces the necessary dose of prednisone by half, thus reducing the long-term side-effects of corticosteroids. MTH is a well tolerated drug, but it requires regular check-up of complete blood count and liver function before the initiation of therapy and during the treatment in order to avoid blood dyscrasia and hepatotoxicity. It is necessary to point out that the positive effects of MTH can be noticed only with cumulative dose, i.e. after at least six months of

treatment. The positive response to MTH therapy has been described in numerous studies and it varies from 60 to 80% of patients. The most serious complication of MTH therapy is its hepatotoxicity. This complication can sometimes be irreversible. The risk certainly increases with the existence of previous liver damage that a patient may have before MTH therapy. The risk of development of hepatotoxicity effects is increased with the existence of diabetes mellitus, alcohol use and obesity. Hepatotoxic effects of MTH are increased with a cumulative dose exceeding 5 grams or in the presence of a kidney disorder, i.e. renal insufficiency. Serum transaminases (aspartate transaminase – AST and alanine transaminase – ALT) must be checked every 6-8 weeks during the therapy. A moderate increase in serum transaminases can be noticed in 30% of the patients on average.

It is usually not necessary to discontinue MTH therapy in these patients because the increased transaminases get normalized spontaneously. However, some patients still require a MTH dose reduction. In case of elevated serum transaminase values in patients with sarcoidosis, liver sarcoidosis must be excluded as a possible cause of transaminase elavations prior to the initiation of therapy.

It is known that sarcoidosis, being a multisystem disease, can affect liver as well, which is manifested in increase of liver enzymes in serum. Simultaneously, 1 mg dose of folic acid a day is recommended in order to prevent macrocytic anemia and possible abnormalities in the liver function, gastrointestinal intolerance and cardiovascular disorders due to the increase in homocysteine concentration in plasma [7,8].

Mycophenolate mofetil (MMF) is an inhibitor of monophosphate dehydrogenase, the enzyme necessary in purine synthesis and weakening of T and B proliferation. It was used before in cutaneous and gastrointestinal sarcoidosis treatment. Recently MMF has proved to be an efficient and well tolerated drug in neurosarcoidosis treatment [9].

Cyclophosphamide is mainly used in severe forms of neurosarcoidosis. In the study performed in 7 patients with neurosarcoidosis treated by cyclophosphamide, the clinical response was recorded in four patients, which was documented by the improvement on images made by MR or by examination of cerebrospinal liquor [10].

Azathioprine is another cytotoxic agent used in sarciodosis treatment. Although its toxicity is similar to MTH toxicity, azathioprine is considered to be significantly potent immunosuppressive agent in treatment of many disorders caused by immune response disorders. The biggest restriction in azathioprine therapy is its potential carcinogenicity. Azathioprine has strong immunosuppressive effect on inflammatory cells (lymphocytes, neutrophils, macrophages,). Muller- Quernheim et al. [11] have demonstrated supreme effects of azathioprine on the increased values of tumor necrosis factor (TNF) from alveolar macrophages in patients with active sarcoidosis. The recommended dose of azathioprine is 2-3 mg/kg. Patients with methyltransferase deficiency have a significantly increased risk for developing neutropenia during azathioprine therapy. Neutropenia is also the most serious toxic effect of azathioprine and is certainly dependant on the total dose of the applied therapy. Regular checking of complete blood count is recommended, i.e. white blood cells, every two to three months in the patients on regular azathioprine therapy. Feeling of nausea and fatigue frequently appear during azathioprine therapy. In a controlled study aimed at giving comparative effects of azathioprine therapy and MTH in the patients with sarcoidosis, azathioprine was significantly more frequently associated with the side-effects displayed by gastrointestinal system [12]. It is not surprising that the feeling of nausea is dependent on the applied dose of azathioprine. Although the toxic effects related to liver or pancreas are rare in azathioprine therapy, regular check-ups of the liver function are recommended. Since regular blood analyses are necessary in this group of patients, simultaneous checking of liver enzymes is also recommended. Carcinogenic effects of azathioprine therapy remain its biggest problem. Studies dealing with organ transplantation issues showed a significantly increased risk of malignancy in patients on azathioprine therapy. The risk is particularly expressed in patients on triple immunosuppressive therapy. On the other hand, studies performed in order to follow the patients on this therapy, but without previous organ transplantation, showed no increased risk of developing malignancy although these patients had been on azathioprine therapy for several years. The efficiency of azathioprine is different in treatment of sarcoidosis patients. In their study, Müller-Quernheim et al. described 11 patients with sarcoidosis who had positive response to therapy, and only three of them had relapse of disease after discontinuation of therapy [12]. On the other hand, Lewis et al. reported positive response in only two out of nine treated patients [13]. Literature data on the efficiency of azathioprine usually state that two thirds of patients have positive response to this therapy. There are no controlled studies on azathioprine efficiency in relation to methotrexate in patients with chronic sarcoidosis. In one study on uveitis associated with sarcoidosis, MTH was the first cytostatic drug used in therapy. The efficiency of this therapy was observed in 36 out of 53 patients; the patients who did not respond to MTH therapy continued azathioprine treatment (21 patients). Only six patients (29%) from the group without the positive therapy response to MTH, responded favorably to mono azathioprine therapy [14, 15].

Chloroquine and hydroxychlorine, which belong to a group of anti-inflammatory drugs, have also shown the efficiency in sarcoidosis treatment.

In a study [16] performed on 12 treated patients, 10 out of 12 of those who had not responded to corticosteroid therapy showed either improvement or stabilization of symptoms. The biggest problem in using this therapy is its ocular toxicity. The toxicity is cumulative and usually reversible. Routine ophthalmologic check-ups are recommended to all patients on this therapy. Less frequent toxic effects of this therapy are skin changes (rash) as well as hepatotoxicity. The inhibition of cytokine release from the alveolar macrophages underlies the anti-inflammatory effect of this therapy. This includes the release of TNF, although other cytokines can be inhibited by the effect of these drugs. The inhibition effect of cytokine release depends on the dose and, accordingly, the described therapy is more efficient where the accumulation of the drug is more intense, e.g. in the skin. In a randomized study on chronic lung sarcoidosis [17], all patients were initially treated with high doses of chloroquine (750 mg a day), and the resulting clinical response was favorable. After these high doses, the patients were divided into two groups, so the first group received low doses of 250 mg a day or they had no therapy. All the patients with sarcoidosis from the group which had been treated by morbostatic doses of chloroquine, had significantly lower number of clinical relapses of disease, i.e. a low percentage of aggravation at the end of this study. This drug has also proved to be very successful in neurosarcoidosis treatment. Here the percentage of improvement is certainly not so dramatically high. One of the possible explanations is low drug concentration in the brain tissue and the lungs in relation to the skin [18].

The aforementioned statements on sarcoidosis therapy suggest that TNF suppression may have a significant role in sarcoidosis treatment. Three known anti-TNF agents used in treatment of immunologically caused diseases in the United States are: antagonist of TNF receptors–etanercept and monoclonal antibodies such as infliximab and adalimumab. The effect mechanism of anti-TNF agents has been studied in other diseases, not exactly in sarcoidosis. Etanercept, being a soluble TNF receptor, binds from the circulation with free TNF, thus preventing TNF from binding with the cell receptors and their activation as well. Infliximab and adalimumab are monoclonal antibodies binding to free TNF in the circulation. Infliximab and adalimubab have the ability of binding even to the surface of cells releasing TNF. Binding is done via immunoglobulin G (IgG) antibodies and may lead to cell lysa. Infliximab was first used in the therapy of refractory lung and skin sarcoidosis in 2001 [19]. Since then there have been numerous published studies reporting the positive effect of infliximab applied to treat not only lung and skin sarcoidosis, but also chronic eye sarcoidosis, upper respiratory airways and muscle sarcoidosis. Etanercept has not proved so efficient in sarcoidosis treatment.

There are more studies dealing with this subject which show modest results in sarcoidosis treatment achieved by this agent. These studies only confirm the equality of placebo response and this therapy agent [20]. Experience is similar in the therapy of Crohn's disease and other inflammatory diseases. The real reason for achieving high concentrations of this drug in circulation may be its intravenous administration, as is the case with infliximab. Toxic effects of anti-TNF therapy are caused by the reaction on the protein component of therapeutics. Etanercept and adalimumab are applied subcutaneously, so here the reaction is local on the application site. Infliximab is applied intravenously so the reactions such as anaphylaxis may be expected. All the above mentioned therapy agents are associated with the increased risk of infection development. The highest risk is the development of granulomatous infections, especially tuberculosis and histoplasmosis. The risk of developing tuberculosis infection is significantly higher in patients treated by infliximab than in those treated by etanercept [21]. In the last ten years, several studies have been performed on infliximab application in neurosarcoidosis treatment and its efficiency in patients who do not respond to corticosteroid therapy [22, 23]. In the sample of ten patients with sarcoidosis, infliximab alleviated the symptoms in 9, including two patients with neurosarcoidosis [24]. Vital capacities improved significantly in 138 patients with chronic lung sarcoidosis [25]. Other studies have shown clinical efficiency of infliximab in patients with cyclophosphamide refractory neurosarcoidosis [26]. Seven patients with refractory neurosarcoidosis treated by combination of MMF and infliximab had clinical improvement without complications [27]. In addition, infliximab can also be used for urgent stabilization of patients with severe condition or in patients with neurosarcoidosis aggravated due to unsuccessful treatment with high doses of corticosteroids [28]. When pharmacotherapy fails in treatment and/or side-effects cannot be tolerated, the alternative treatment is radiotherapy (RT) of CNS. RT functions on the principle of locating and destroying the cells, such as macrophages and lymphocytes which are directly involved in granuloma forming and which are metabolically active. Recent studies have shown minimal and moderate efficiency of RT in persistent sarcoidosis of CNS. In the biggest study performed so far, researchers treated four patients with neurosarcoidosis resistant to pharmacotherapy by radiotherapy. The complete remission of most of the symptoms was achieved in one patient, and the partial and minimal remission was achieved in two patients and one patient, respectively [29]. In another study, two out of three patients treated by RT had almost complete remission of symptoms [30]. Since the patients in all previous treatments received different doses of gray (Gy) during radiation, the comparison of radiation doses was not possible. Further research is

necessary which would be oriented towards the optimal RT strategy. As mentioned before, the histopathological finding of the involved tissue remains the gold standard in neurosarcoidosis diagnostics. From the therapeutic standpoint, neurosurgery is indicated only when pharmacology is without effect, or in patients with urgent or life threatening conditions. Complications, such as severe hydrocephalus and increased intracranial pressure, can be treated by ventriculoperitoneal shunt. Afterwards, surgical resection of the white mass may be taken into consideration in life threatened patients [31].

Conclusion

It is the variety of forms of this disease, the absence of diagnostic criteria and different and non standardized therapy that make this treatment difficult. In our country, there is a lack of controlled studies on the treatment of these patients, particularly on the application of new immunosuppressive drugs. In spite of advances in pharmacology and radiology, it is necessary to develop better diagnostic strategies in order to set optimal therapeutic approach.

References

1. Škodrić-Trifunović V, Vučinić V, Simić-Ogrizović S, Stević R, Stjepanović M, Ilić K, et al. Mystery called sarcoidosis: forty four years follow up of chronic systemic disease. Srp Arh Celok Lek. 2012;140(11-12):768-71.

2. Savić M, Stjepanović M, Mihailović-Vučinić V, Gvozdenović B, Filipović S, Mujović N. Sarkoidoza: novine u dijagnostici. Med Pregl 2013;66(Suppl 1):22-5.

3. Stjepanović M. Vanplućne komplikacije plućne sarkoidoze-neurosarkoidoza: evaluacija, terapija i komplikacije zahvaćenosti nervnog sistema (specijalistički rad). Beograd: Medicinski fakultet, Univerzitet u Beogradu; 2013.

4. Stjepanović M, Mihailović-Vučinić V, Dudvarski-Ilić A, Videnović-Ivanov J, Škodrić-Trifunović V, Popević S. Kliničke manifestacije neurosarkoidoze. Med Pregl 2013;66(Suppl 1):54-9.

5. Filipović S, Mihailović-Vučinić V, Omčikus M, Videnović-Ivanov J, Stjepanović M, Šumarac Z. Hitotriozidaza: potencijalni biomarker u sarkoidozi. Med Pregl 2013;66(Suppl 1):26-8.

6. Lower EE, Broderick JP, Brott TG, Baughman RP. Diagnosis and management of neurological sarcoidosis. Arch Intern Med 1997;157(16):1864-8.

7. Vucinic-Mihailovic V. What is the future of Methotrexate in sarcoidosis? A study and review. Curr Opin Pulm Med 2002;8:470-6.

8. Baughman RP, Winget DB, Lower EE. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. Sarcoidosis Vasc Diffuse Lung Dis 2000;17(1):60-6.

9. Chaussenot A, Bourg V, Chanalet S, Fornari JM, Lebrun C. Neurosarcoidosis treated with mycophenolate mofetil: two cases. Rev Neurol (Paris). 2007;163(4):471-5.

10. Doty JD, Mazur JE, Judson MA. Treatment of corticosteroid-resistant neurosarcoidosis with a short-course cyclophosphamide regimen. Chest 2003;124(5):2023-6.

11. Müller-Quernheim J, Kienast K, Held M, Pfeifer S, Costabel U. Treatment of chronic sarcoidosis with an azathioprine/prednisolone regimen. Eur Respir J 1999;14(5):1117-22.

12. McKendry RJ, Cyr M. Toxicity of methotrexate compared with azathioprine in the treatment of rheumatoid arthritis: a case-control study of 131 patients. Arch Intern Med 1989;149(3):685-9.

13. Jewis SJ, Ainslie GM, Bateman ED. Efficacy of azathioprine as second-line treatment in pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 1999;16(1):87-92.

14. Baughman RP, Lower EE, Bradley DA, et al. Use of cytotoxic therapy for chronic ophthalmic sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 1999;16:S17.

15. Hoitsma E, Drent M, Sharma OP. A pragmatic approach to diagnosing and treating neurosarcoidosis in the 21 st century. Curr Opin Pulm Med 2010;16:472-9.

16. Sharma OP. Effectiveness of chloroquine and hydroxychloroquine in treating selected patients with sarcoidosis with neurological involvement. Arch Neurol. 1998;55(9):1248-54.

17. Kalish RS, Koujak S. Minocycline inhibits antigen processing for presentation to human T cells: additive inhibition with chloroquine at therapeutic concentrations. Clin Immunol 2004;113(3):270-7.

18. Mihailović-Vučinić V, Stjepanović M, Videnović-Ivanov J, Gvozdenović B, Filipović S, Dudvarski-Ilić A. Nesteroidna terapija sarkoidoze. Med Pregl 2013;66(Suppl 1):60-6.

19. Baughman RP, Lower EE. Infliximab for refractory sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2001;18(1):70-4.

20. Baughman RP, Lower EE, Bradley DA, Raymond LA, Kaufman A. Etanercept for refractory ocular sarcoidosis: results of a double-blind randomized trial. Chest 2005;128(2):1062-47.

21. Banse C, Bisson-Vaivre A, Kozyreff-Meurice M, Vittecoq O, Goëb V. No impact of tumor necrosis-factor antagonists on the joint manifestations of sarcoidosis. Int J Gen Med 2013; 6:605-11.

22. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijsers RG, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. Eur Respir J 2014;43:602-9.

23. Sollberger M, Fluri F, Baumann T, Sonnet S, Tamm M, Steck AJ, et al. Successful treatment of steroid-refractory neurosarcoidosis with infliximab. J Neurol 2004;251(6):760-1.

24. Doty JD, Mazur JE, Judson MA. Treatment of sarcoidosis with infliximab. Chest 2005;127:1064-71.

25. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 2006;174(7):795-802.

26. Sodhi M, Pearson K, White ES, Culver DA. Infliximab therapy rescues cyclophosphamide failure in severe central nervous system sarcoidosis. Respir Med 2009;103(2):268-73.

27. Moravan M, Segal BM. Treatment of CNS sarcoidosis with infliximab and mycophenolate mofetil. Neurology 2009;72:337-40.

28. Stern BJ, Aksamit A, Clifford D, Scott TF. Neurosarcoidosis study group. Neurologic presentations of sarcoidosis. Neurol Clin 2010;28(1):185-98.

29. Menninger MD, Amdur RJ, Marcur RB Jr. Role of radiotherapy in the treatment of neurosarcoidosis. Am J Clin Oncol 2003;26:e115-e118.

30. Kang S, Suh JH. Radiation therapy for neurosarcoidosis: report of three cases from a single institution. Radiat Oncol Invest. 1999;7:309-12.

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BIBLID.0025-8105:(2014):LXVII:5-6:161-166.

31. Stjepanović M, Mihailović-Vučinić V, Jovanović D, Mijajlović M, Škodrić-Trifunović V, Videnović-Ivanov J. Radiological presentation of neurosarcoidosis. Med Pregl 2014;67(1-2):24-7.

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WHAT KIND OF MILK CAN PREVENT INFANT'S SIDEROPENIC ANEMIA – COMPARATIVE STUDY

KOJOM VRSTOM MLEKA JE MOGUĆE PREVENIRATI NASTANAK SIDEROPENIJSKE ANEMIJE KOD ODOJČADI – KOMPARATIVNA STUDIJA

Olgica MILANKOV, Milena BJELICA and Radojica SAVIĆ

Summary

Introduction. The most common cause of sideropenic anemia in infants, during the period of their fast growth and development, is inadequate nutrition or insufficient intake of food rich in iron. The aim of this paper is to provide the insight into the problem of anemia and to emphasize nutrition as an important etiologic factor in the onset and prevention of anemia in infants. **Material and Methods.** Two retrospective studies were conducted at the Institute for Child and Youth Healthcare of Vojvodina, Department for Infant and Small Children's Pathology. The first study covered the period of eight years (1988-1995), and it included a total of 507 children, aged 1-24 months. The second study covered the period of two years (2010-2011) and a total of 290 children aged 1-12 months were included. The diagnosis of anemia was made according to clinical examination or after taking routine laboratory tests. According to the criteria of the World Health Organization, all children were divided into those with severe, moderate or mild anemia. **Results.** Out of 507 children examined in the first study, 333 (65.68%) were breastfed, while 174 (34.32%) had never been breastfed. In the second study, 206 (71.03%) out of 290 children were breastfed, while 56 (19.31%) had never been breastfed. In both studies the highest percentage of children breastfed for the longest period was among children with mild form of anemia, while the children who were breastfed for the shortest period had severe anemia. In addition, the highest percentage of anemic children was supplementary fed with cow's milk in both studies. **Conclusion.** Short natural diet, early introduction of supplementation and choice of milk could be determining factors in the development and manifestation of anemia.

Key words: Milk; Infant; Anemia, Iron-Deficiency; Breast Feeding; Infant Nutritional Physiological Phenomena; Risk Factors; Hemoglobins; Iron

Sažetak

Uvod. Najčešći uzrok nastanka sideropenijske anemije kod odojčadi, tj. u periodu ubrzanog rasta i razvoja, jeste nepravilna ishrana ili nedovoljan unos gvožđa hranom. Cilj rada je da pruži uvid u problem pojave anemije i da potencira ishranu kao važan etiološki faktor za nastanak, odnosno prevenciju anemije u odojčadskom uzrastu. **Materijal i metode.** Retrospektivnim studijama koje su sprovedene u Institutu za zdravstvenu zaštitu dece i omladine Vojvodine, na Odeljenju za patologiju odojčeta i malog deteta, obuhvaćeno je 507 dece uzrasta 1–24 meseca u periodu od 1988. do 1995. godine i 290 dece uzrasta 1–12 meseci tokom 2010. i 2011. godine. Dijagnoza malokrvnosti postavljena je na osnovu kliničkog pregleda ili nakon uzimanja rutinskih laboratorijskih nalaza. Sva deca su podeljena, prema kriterijumima Svetske zdravstvene organizacije, na decu sa teškom, umerenom ili lakom anemijom. **Rezultati.** U prvoj studiji, od ukupnog broja dece, 333 (65,68%) dece bilo je dojeno, dok 174 (34,32%) nikada nije bilo na prirodnoj ishrani. U drugoj studiji, od ukupnog broja dece, 206 (71,03%) bilo je dojeno, dok 56 (19,31%) nikada nije sisalo. U obe studije, najveći procenat najduže dojene dece bio je među decom sa lakim oblikom anemije, dok su najkraće dojena deca bila sa teškim oblikom anemije. Isto tako je u obe studije najveći procenat teško anemične dece dohranjivan kravljim mlekom. **Zaključak.** Kratkotrajna prirodna ishrana, rano započeta dohrana i izbor mleka mogli bi biti determinišući faktori u nastanku i ispoljavanju anemije.

Ključne reči: Mleko; Odojče; Sideropenijska anemija; Dojenje; Fiziologija ishrane odojčeta; Faktori rizika; Hemoglobina; Gvožđe

Introduction

The problem of iron deficiency anemia, although being a multi-interesting topic, is still not enough talked about. According to the reports of the World Health Organization (WHO), anemia is "... one of the most common diseases of undernourishment in the world...". Thirty percent of the

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Abbreviations
HGB – hemoglobin
WHO – World Health Organization

world's population, that is 1.3 billion people have iron deficiency; the percentage being 43%, 51% and 37% in the children of pre-school population, women and children of school-age, respectively [1].

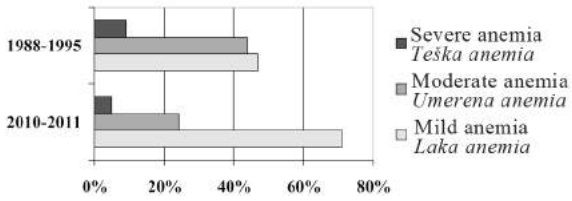
Iron deficiency anemia is the most common hematological disorder among infants encountered in everyday practice. Although many studies have tackled this problem, it has not lost any of its relevance over time, since the factors causing iron deficiency anemia are still present in our environment [2]. Three quarters of cases of anemia in the first two years of life are caused by iron deficiency. During this period of life there is an increased need for iron, combined with iron loss and undernourishment, which is why this age is known as "hematologically vulnerable age". According to some authors, anemia affects children from poor social, hygienic and cultural backgrounds. Studies indicate that iron deficiency anemia has a growing trend, especially in economically deprived conditions, in the countries where iron is almost completely absent in the diet of population, or in countries where the diet is reduced because of poverty, war, inadequate agricultural policy or wrong social doctrine [2-4]. However, this type of anemia is also present in rich, industrialized countries, where it is caused by the consumption of refined and technologically processed food, which is rich in energy but less valuable in quality. A certain amount of iron and other elements involved in the synthesis of hemoglobin is lost in the process of food production. Therefore, many countries started iron supplementation of different types of food in order to compensate for inadequate food intake of iron. Flour, salt, cereals, rice and various spices are enriched with iron to prevent the development of anemia [5]. The most often cause of sideropenic anemia in infants during the period of their fast growth and development is incorrect nutrition or insufficient intake of food rich in iron. This is the reason why this anemia falls into the group of nutritional anemias. The prevalence of anemia in this vulnerable period is more than 30% [2, 6]. It is well known that both human and cow's milk contain insufficient amount of iron (1.5 mg/L and 0.5 mg/L, respectively). Keeping in mind the average absorption of iron of about 10%, it is to be expected that infant's nutrition will be deficient in iron, because milk is the main and basic food for an infant. Due to the increased need for iron intakes at this age, and because of the relatively low value of iron in foods, even in its most perfect form, with a proper diet, the quantity of iron consumed is not enough for most children to prevent the development of nutritional anemia. Therefore, iron deficiency in infants is often caused by the use of milk diet without added iron or with inadequate addition of iron [2, 7, 8]. During the first year of life, infants born at term require 160 mg, and premature infants about 240 mg of iron to meet the needs of erythropoiesis. Approximately 50 mg of iron is provided from the disintegrated erythrocytes that are physiologically present in the first week of life. The rest of iron must be provided through food. Human and cow's milk contain small amounts of iron (about 1 mg Fe/L). However, iron in human milk is absorbed better (49% vs. 10% in cow's milk). This is the reason why infants who are breastfed for the first six months of life have higher levels of serum ferritin and saturation of transferrin greater than children fed with cow's milk. In addition, in children who were fed with unmodified cow's milk, iron deficiency can occur not only because of scarce iron content of milk and reduced absorption of iron from milk, but because of possible bleeding from gastrointestinal organs [2, 8].

The aim of this paper is to provide at least a partial insight into the problem of anemia and to emphasize nutrition as an important etiologic factor for the development of anemia in infants.

Material and Methods

We compared two retrospective studies conducted at the Institute of Child and Youth Healthcare of Vojvodina, Department for Infant and Small Children's Pathology in different periods of time. The first one was conducted from 1988 to 1995 and included 507 hospitalized children aged from 1 to 24 months. The second study was conducted from 2010 to 2011 and included 290 hospitalized children aged from 1 to 12 months. The children were referred to the Institute with different diagnoses, and diagnosis of anemia was based on the typical history, physical examination and the results of routine laboratory tests. All laboratory data were obtained in the laboratory of the Institute for Child and Youth Healthcare of Vojvodina in Novi Sad and the following instruments were used: automatic biochemical system Hitachi 704, hematological counter MS9 (Melet Schlosing), ISE analyzer AVL 983 Scales Sartorius 6000, centrifuge Hermle 9 and mixer Hanna 4. Hematological parameters were obtained by using the hematological instrument MS9. This instrument determines the number of erythrocytes with the help of volumetric impedance (Baker's principle). The principle of counting and determining the size of cells is based on the difference in the conductivity of pure cells and diluents in which the cells are suspended. The level of hemoglobin (HGB) was determined by the standard cyanmethemoglobin method.

According to the criteria of the WHO, the entire population of children was divided into three groups by the values of hemoglobin, biochemical and hematological status. These groups were as follows: 1) children with severe anemia (hemoglobin level below 70 g/L), 2) children with moderate anemia (hemoglobin level between 70 and 90 g/L), and 3) children with a mild form of anemia (hemoglobin 90 to 110 g/L). All children were analyzed according to



Graph 1. The intensity of anemia
Grafikon 1. *Intenzitet anemije*

the type of milk feeding and the intensity of anemia. The collected data were processed by the appropriate modern statistical methods.

Results

The first retrospective study included 507 children aged 1 to 24 months with sideropenic anemia who were hospitalized at the Institute of Child and Youth Healthcare of Vojvodina, Department for Infant and Small Children's Pathology during the period of eight years. Among the examined children, 9.07% suffered from severe anemia, 43.98% had a moderate form, while 46.94% had a mild form of anemia. The second retrospective study included 290 children aged form 1 to 12 months with sideropenic anemia who were hospitalized at the Institute for Child and Youth Healthcare of Vojvodina, Department of Infant and Small Children's Pathology during the period of two years. Among the examined children, 4.82% suffered from severe anemia, 24.13% had a moderate form, while 71.03% had a mild form of anemia (**Graph 1**). Out of 507 children examined in the first study, 61.74% were undernourished. The analysis of the collected data shows that the highest percentage of underweight children had severe anemia (15.22%). Children who had been well-fed developed a mild form of anemia (48.74%). Out of 290 children examined in the second study, 23.44% were undernourished. Body weight in children with a mild form of anemia was significantly higher compared to children with moderate and severe forms of anemia. In the first study, 333 children (65.68%) were breastfed, while 174 (34.32%) had never been breastfed. Further analysis of the data shows that 102 children (19.92%) were breastfed only. The percentage of breastfed infants in different grades of anemia was as follows: 50% (severe), 64.13% (moderate), and 70.17% (mild). In the second study, 206 children (71.03%) were breastfed, while 56 (19.31%) had never been breastfed (**Table 1**). Further analysis of the data shows that 83 children (28.62%) were breastfed only. The percentage of breastfed infants in different grades of anemia was as follows: 57.1% (severe), 62.9% (moderate), and 74.8% (mild). In the first study, 223 children (43.98%) were fed only complementary food, while 141 children (27.81%) were both breastfed and given complementary food. In the second study, 156 children (53.79%) were fed only complementary food, while 32 children (11.03%) were both breastfed and given complementary food **(Table 2)**. Among all the children examined in the first study, 2/3 diluted cow's milk was given as complementary food to 34.52% children, undiluted cow's milk and goat's milk was given to 5.33% and 4.14% children, respectively; whereas 28.4% of children were fed different industrial milk formulas. In the second study, the highest percentage of children were fed industrial milk formulas (50%), 25.64% were fed 2/3 diluted cow's milk, while ½ cow's milk, undiluted cow's milk and goat's milk were rarely used.

Our studies showed that there were significant differences among children with anemia of varying intensity with respect to breastfeeding. The highest percentage of children who were breastfed for the longest period were found to be the children with the mild form of anemia, while the children who were breastfed for the shortest period had severe anemia. In addition, there was a significant difference in terms of complementary feeding among the three groups of children in both studies. The results revealed that cow's milk was given as supplementary food to 56.52% of the seriously anemic

Table 1. Type of children's diet
Tabela 1. *Način ishrane dece*

Diet *Način ishrane*	Breastfed/*Dojeno*		Not breastfed/*Nedojeno*	
	I study/*I studija*	II study/*II studija*	I study/*I studija*	II study/*II studija*
N	333	206	174	56
%	65,68	71,03%	34.32	19,31%

Table 2. Type of children's nutrition
Tabela 2. *Vrsta ishrane dece*

Type of nutrition *Vrsta ishrane*	Breastfed only *Prirodna*		Complementary fed *Veštačka*		Breastfed and complementary fed *Dohrana*	
	I	II	I	II	I	II
N	102	83	223	156	141	32
%	19,92	28,62	43,98	53,79	27,81	11,03

children in the first study and 64.3% in the second study, 43.05% of moderately anemic children in the first study and 51.4% in the second study and 42.44% of the children with a mild form of anemia in the first study and 53.9% in the second study.

Discussion

Anemia in children under two years of age is a common health problem due to the fact that their growth requires a high intake of iron which is usually not provided by their diet [9]. By comparing these two studies it was observed that the percentages of the children with severe and moderate form of anemia were twice lower in the second study, while the percentage of the children with a mild form of anemia almost doubled compared to the first study. Anemia is usually caused by several associated factors, rather than individual ones. The important factors are the impact of environment, socio-economic factors, habits in the family, especially the ones related to the mother, certain individual characteristics (gender, time and way of delivery, birth weight and associated diseases) as well as the child's diet. The level of nutrition is certainly correlated with different quality of alimentary deficit. In infancy, the overall mental and physical development depends primarily on the diet, and the diet relies almost entirely on the attitude, perception, cultural and health education of the mother [2]. Most of the authors think that children suffering from anemia due to iron deficiency tend to have lower body weight [10, 11]. Other authors, however, believe that better nutritional status does not necessarily mean higher hemoglobin values, that these two factors are not directly related [12–14]. According to the data from both studies, the body weight of children with a mild form of anemia was significantly higher compared to the children with moderate and severe forms of anemia.

It is well known that milk is a prototype of food poor in iron. In the first six months of life, milk is the basic food. It has been shown that the absorption of iron from breast milk is much higher compared to cow's milk. Fortified milk formula contains the highest amount of iron, and its use is recommended in the diet of children under one year of age. Literature indicates that anemia has the highest prevalence in children fed with cow's milk, while it is less common in breastfed children. Prevalence of anemia is the lowest in children fed with adapted milk formulas. These are the reasons why the WHO recommends that infants who are not breastfed should not get diluted or unmodified cow's milk until the age of one. Iron rich baby formulas are therefore highly recommended [2, 15–19]. The comparison of these two studies shows that the children from the second study sample were breastfed in a slightly higher percentage (71.03%) compared to the first study (65.68%). In both studies, the children with a mild form of anemia were breastfed in the highest percentage.

In 1928, Helen McKay concluded that anemia was common in infants on complementary diet, but did not know the exact reason for this phenomenon. Eight decades later, studies suggested that the results of her research about the dependence of infant feeding and the development of anemia could be accepted with only minor adjustments. Therefore, she is considered to be the founder of modern study of anemia caused by iron deficiency. At that time, breastfeeding lasted much longer than today, and the results of her study showed that the hemoglobin concentration was higher in breastfed children. Complementary feeding at that time was actually cow's milk powder, without the addition of iron and other minerals or vitamins. She identified the real cause of this phenomenon to be better absorption of iron from human milk (50%) than from cow's milk (10%). Approximately one third of the infants who were fed with cow's milk showed blood loss through stool, which could be another factor in developing anemia due to iron deficiency. In addition, she suggested a definition of anemia, which is very similar to the one given by the WHO 40 years later [2, 15, 20].

As for the kind of milk introduced during the complementary feeding, 2/3 diluted cow's milk was mainly used in the first study, whereas in the second study, the use of industrial milk formulas was dominant, which is in accordance with the recommendations of the WHO. Frequent use of industrial milk formulas in nutrition of children, that was observed in the second study, may indicate a higher level of health education of mothers and may suggest the increase in socio-economic standards of the population compared to the previous periods [2, 5].

Inadequate nutrition is a significant problem, especially in this vulnerable population. Since deficient diet has a negative impact on general health of population, anemia is considered to be not only a health problem, but a social problem as well [2]. The analysis of the results of our studies shows that children who used cow's milk as complementary food had severe form of anemia. Short-termed natural nutrition, early supplementation, and choice of milk could be determining factors in the development and manifestation of anemia [2, 8, 16, 19, 20]. Breastfeeding for more than six months of life without the addition of iron, early feeding with unmodified cow's milk or solid foods can be the causes of sideropenic anemia. It can also be caused if non-fortified baby formulas are used longer than four months without introducing other mixed foods. Children who are born at term and breastfed for the first six months of life are not at risk in terms of iron deficiency. After this age, if the child is not breastfed, supplementary diet should include industrial milk infant formula enriched with iron. At the same time, it is recommended to introduce mixed foods. If solid foods are introduced to children who are still

being breastfed, it can improve the bioavailability of iron from human milk [21].

Conclusion

Anemia due to iron deficiency effects people of all ages and from all economic groups, although it is more common among the younger population groups, particularly infants and young children aged from 1 to 24 months. It is particularly common among people with insufficient nutrition. From what has been said, one can only imagine the extent to which iron deficiency anemia is widespread in our country and worldwide. What is yet undiscovered is the percentage of patients who are not registered and are thus not treated.

The comparison of two retrospective studies shows that the percentage of children with severe and moderate forms of anemia had decreased through the years and it was twice lower in the second study, while the percentage of children with a mild form of anemia had increased, in fact it doubled in the second study. Both studies confirmed the significant role of breastfeeding in prevention of sideropenic anemia. Also, it has been observed that the diet of children changes through the years and that diluted 2/3 cow's milk, which was previously frequently used in complementary feeding of children, is now often replaced by adapted milk formulas, which is in accordance with the World Health Organization recommendations.

Given the high prevalence of anemia in infants (30% of children treated at the Department), all the necessary social, economic and educational measures muste be taken to correct the diet and to ensure optimal nutritional and energy needs, as well as the needs for iron, and thus prevent development of sideropenic anemia in this age group.

References

1. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B.Worldwide prevalence of anaemia. WHO vitamin and mineral nutrition information system, 1993-2005. Public Health Nutr 2009;12(4):444-54.

2. Milankov O. Faktori rizika kod sideropenijske anemije odojčadi (doktorska disertacija). Beograd: Univerzitet u Beogradu; 2003.

3. Diaz JR, Cagigas A, Rodriguez R. Micronutrient deficiencies in developing and affluent countries. Eur J Clin Nutr 2003;57(1):70-2.

4. Dos Reis MC, Nakano AM, Silva IA, Gomes FA, Pereira MJ. Prevalence of anemia in children three to 12 months old in a health service in Ribeirão Preto, SP, Brazil. Rev Lat Am Enfermagem 2010;18:792-9.

5. Marx JJM. Iron deficiency in developed countries: prevalance, influence of lifestyle factors and hazards of prevention. Eur J Clinic Nutr 1997;51(8):491-4.

6. Duque X, Flores-Hernández S, Flores-Huerta S, Méndez-Ramírez I, Muñoz S, Turnbull B, et al. Prevalence of anemia and deficiency of iron, folic acid, and zinc in children younger than 2 years of age who use the health services provided by the Mexican Social Security Institute. BMC Public Health 2007;30:345.

7. Perišić V, Borislav J. Pedijatrija. Beograd: Medicinski fakultet; 2010.

8. Milankov O. Ishrana odojčadi: iskustva, novi trendovi i preporuke. Med Pregl 2013;66:5-10.

9. World Health Organization. The world health report 2002: reducing risks, promoting healthy life. Geneva: WHO; 2002.

10. Wise J. Daily iron during pregnancy improves birth weight. BMJ 2013;346:f3997.

11. Amrutha Veena K, Kowsalya S, Kothandapani S. Micronutrient malnutrition profile of infants in South India. J Hum Nutr Diet 2014;27(1):54-7.

12. Suskind DL. Nutritional deficiencies during normal growth. Pediatr Clin North Am 2009;56(5):1035-53.

13. Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ. A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. J Pediatr 2003;143(5):582-6.

14. Long H, Yi JM, Hu PL, Li ZB, Qiu WY, Wang F, et al. Benefits of iron supplementation for low birth weight infants: a systematic review. BMC Pediatr 2012;12:99.

15. Elalfy MS, Hamdy AM, Abdel Maksoud SS, Abdel Megeed RI. Pattern of milk feeding and family size as risk factors for iron deficiency anemia among poor Egyptian infants 6 to 24 months old. Nutr Res 2012;32:93-9.

16. Dube K, Schwartz J, Mueller MJ, Kalhoff H, Kersting M. Iron intake and iron status in breastfed infants during the first year of life. Clin Nutr 2010;29:773-8.

17. Sawasdivorn S, Taeviriyakul S. Are infants exclusively breastfed up to 6 months of age at risk of anemia? J Med Assoc Thai 2011;94:178-82.

18. Oliveira MA, Osório MM. Cow's milk consumption and iron deficiency anemia in children. J Pediatr (Rio J) 2005; 81:361-7.

19. Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. Nutr Rev 2011;69:37-42.

20. Hopkins D, Emmett P, Steer C, Rogers I, Noble S, Emond A. Infant feeding in the second 6 months of life related to iron status: an observational study. Arch Dis Child 2007;92:850-4.

21. Baker R, Greer F. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics 2010;126:1040.

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EFFECTS OF PHTHALIC ACID ESTERS ON FETAL HEALTH

UTICAJ ESTARA FTALNE KISELINE NA FETALNO ZDRAVLJE

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Summary

Introduction. Phthalates are synthetic industrial compounds capable of disrupting endocrine system. Effects of phthalates depend on dosage, duration of action and stage of development of the individual, thus making the fetus, newborn, and children at puberty the most vulnerable groups. **Metabolism of Phthalates:** Metabolism of these compounds consists of at least two steps: hydrolysis and conjugation. They are mainly excreted in urine, with a low percent being excreted through feces. **Exposure to Phthalates.** Exposure to the effects of phthalates begins at the intrauterine stage since the phthalates pass through the placental barrier. Phthalates may be found in plastic products, toys, medical equipment, industrial materials, food, and clothes. **Determination of Phthalate Levels in Humans.** Urine is the best sample for evaluating phthalate levels in humans because of rapid phthalate metabolism and high concentrations of metabolites in the urine. **Fetal Testicular Dysgenesis Syndrome:** Fetal testicular dysgenesis syndrome involves disorders of male genital tract such as shortened anogenital distance, hypospadia, cryptorchidism, malformations of seminal vesicles, prostate, epididymis and it results from the harmful effects of phthalates. **Other Effects of Phthalates on Health.** Negative effects of phthalates on female health are mostly reflected in anovulation, premature puberty, changes in duration of pregnancy. There is a possible effect on neurocognitive development, occurrence of allergies, asthma, testicular carcinoma, hepatic and renal damages, insulin resistance and obesity, thyroid dysfunction. **Conclusion**. Further studies are needed to establish the safe phthalate concentration in certain products and to determine more negative consequences of exposure to phthalate.

Key words: Phthalic Acids; Fetus; Endocrine Disruptors; Plastics; Gonadal Dysgenesis; Insulin Resistance

Sažetak

Uvod. Ftalati su sintetska industrijska jedinjenja koja imaju sposobnost da remete funkciju endokrinog sistema. Njihovi efekti zavise od doze, dužine dejstva i razvojnog stadijuma jedinke, te su fetus, novorođenče i deca u pubertetu najugroženije kategorije. **Metabolizam ftalata.** Metabolizam ftalata odvija se u najmanje dva koraka − hidroliza i konjugacija. Ekskrecija najvećeg dela ftalata obavlja se urinom, međutim manji procenat se izlučuje fecesom. **Izloženost ftalatima.** Izloženost ftalatima počinje još intrauterino, pošto oni slobodno prolaze placentarnu barijeru. Prisutni su u plastičnim prozvodima, igračkama, medicinskim instrumentima, industrijskim materijalima, hrani, odeći. **Određivanje nivoa ftalata u ljudskom organizmu.** Urin je materijal izbora za određivanje nivoa ftalata, zbog njihovog brzog metabolizma i visokih koncentracija metabolita ftalata u urinu. **Sindrom fetalne testikularne dizgenezije** nastaje kao posledica štetnog dejstva ftalata; podrazumeva anomalije genitalnog trakta: skraćena ano-genitalna distanca, hipospadija, kriptorhizam, malformacije semenih vezikula, prostate, epididimisa. **Drugi efekti ftalata na zdravlje.** Kod osoba ženskog pola negativan uticaj ftalata ogleda se u anovulaciji, preranom pubertetu, promenama u dužini trajanja trudnoće. Smatra se da neželjeni efekti ftalata mogu da se ispolje i kroz neurokognitivne poremećaje, pojavu alergija, astmu, karcinom testisa, oštećenja jetre i bubrega, insulinsku rezistenciju i gojaznost, tiroidnu disfunkciju. **Zaključak.** Neophodna su dalja istraživanja kojima bi se odredile bezbedne koncentracije ftalata, ali i da bi se uočili i do sada neprepoznati neželjeni efekti ftalata na ljudsko zdravlje.

Klučne reči: Ftalna kiselina; Fetus; Endokrina disrupcija; Plastika; Gonadna disgenezija; Insulinska rezistencija

Introduction

Interest in chemical matters that disrupt the endocrine system work - endocrine disrupting chemicals (EDCs) has been increased over the last several years. EDCs may affect the synthesis, secretion, mechanism of action, metabolism and elimination of hormones in humans and animals, with harmful health consequences.

Phthalates, esters of 1,2-dicarboxylic acid - phthalic acid, are synthetic industrial chemical compounds which were introduced in 1920. Ever since 1933 and the synthesis of di (2-ethylhexyl) phthalates (DEHP), the phthalates have been the most common chemical compounds with the possibility to disrupt the endocrine system [1]. The effect of phthalates depends on dosage, duration of action and stage of the development of the individual, thus

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Abbreviations

EDCs – endocrine disrupting chemicals
DEHP – di (2-ethylhexyl) phthalates
MEHP – mono(ethyl-hexyl) phthalate
BBzP – butyl benzyl phthalate
DBP – di-n-butyl phthalate
DINP – di-isononyl phthalate
DIDP – di-isodecyl phthalate
DMP – di-methyl phthalate
DnOP – di-n-octyl phthalate
MEP – mono-ethyl phthalate
MBP – mono-butyl phthalate
MMP – mono-methyl phthalate

making the fetus, newborn, and children at puberty the most vulnerable groups [2]. According to the study of Latini et al. from 2003, the exposure to the effects of phthalates begins at the intrauterine stage since the phthalates pass through the placental barrier; another important issue in relation to the phthalates, i.e. EDCs, is that intrauterine exposure may not be manifested until adolescence or even later [1]. It is also known that exposure to phthalates may be manifested only as disorders of the next generations (through modification of factors that regulate gene expression) [1]. Because of the known harmful effects, the European Union countries as well as our country have limited the use of DEHP, butyl benzyl phthalate (BzBP) and di-n-butyl phthalate (DBP) in the production of children's toys and items intended for the child care, while the restrictions on the use of di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP) and di-n-octyl phthalate (DNOP) apply only to the manufacturers of toys and items intended for child care that children can put in the mouth [3]. Application of DEHP and DBP and BzBP is not permitted in cosmetic production in the European Union because of reproductive toxicity [4].

Metabolism of Phthalates

After they enter the body, phthalates are subjected to hydrolysis and conjugation [4]. Monoestar phthalates are created by hydrolysis. *In vivo* and *in vitro* studies proved monoester phthalates to be biologically more active than their diesters [5,6]. Short chain phthalates are excreted in the urine as monoester phthalates, and the long chain phthalates are subjected to further metabolism in terms of hydroxylation and oxidation after which they are excreted in urine and feces [7, 8]. Their biological half-life is short, more than 60% is excreted in 24 hours [1, 9].

Exposure to Phthalates

Low molecular weight phthalates, for example di-methyl phthalate (DMP), DBP, are present in cosmetic products (nail polish, perfumes, facial creams, shampoos, body lotions...), while high molecular weight phthalates, for example DEHP, BBzP, DNOP, DINP, DIDP, are present in plastic containers, adhesives, clothes of raincoat type and plastic products with polyvinyl chloride which is added in order to improve flexibility [4]. Phthalates are also found in medical instruments such as central venous and urinary catheters, as well as in packaging for total parenteral nutrition and intravenous infusion. They are also present in some medications [11]. Foodstuffs, such as cereals, bread, biscuits, cakes, nuts, oils and fats, can be found in packaging made of plastics containing DEHP, DBP and DEHP and di-isobutil phthalate (DIBP), and thus they are in contact with phthalates [12].

Toys are another important source of exposure to phthalate (soothers, teethers, bath toys), and they can enter the body either orally, or by inhalation, through skin and parenterally [1,10].

Determination of Phthalate Levels in Humans

Phthalates do not tend to bioaccumulate and their half-life is less than 24 hours [1,10]. There have been attempts at determining phthalate levels in saliva, serum, seminal fluid, meconium and placenta but, the validation of these procedures have shown that phthalates are excreted in a very small percentage in this way [13, 14]. Urine, maternal milk, serum and amniotic fluid are most frequently used nowadays as material to assess the presence of phthalates in the body [15]. Urine was proven to be the best sample in epidemiological studies in regards to the rapid metabolism of phthalates and high concentrations of the metabolites in the urine. Further advantages of urine as material for determining levels of phthalates is that it can be collected in a noninvasive way and may reflect exposure to phthalates in the last few days, even weeks [16, 17]. In all the above mentioned samples, the level of monoesters, i.e. phthalates metabolites, are determined because the level of monoesters is higher than the level of diesters of phthalic acid, and the contamination of the sample by ubiquitous diesters during the collection, storage and analysis itself is avoided [18].

Fetal Testicular Dysgenesis Syndrome

In the last fifteen years, a number of studies on experimental animals (rats) have proven that phthalates, especially DEHP, DBP, BBzP, when acting in a critical period of the development of genital tract, lead to disturbances in androgen-signaling pathway [18,19]. In almost all previous studies, the anti-androgen effect of phthalates in newborn males was examined, but it was also shown that the negative effects of phthalates on female health are reflected in anovulation, premature puberty, changes in the duration of pregnancy and other disorders [2, 10].

Fetal testicular dysgenesis syndrome ("phthalate syndrome" in rodents) involves disorders of male genital tract in terms of shortened anogenital distance, hypospadia, cryptorchidism, malformations

Table 1. Phthalate diesters and their metabolites (taken from Frederiksen H, Skakkebek NE, Andersson AM. Metabolism of phthalates in humans, Mol Nutr Food Res 2007;51:899-911.)
Tabela 1. *Diestri ftalata i njihovi metaboliti (preuzeto iz Frederiksen H, Skakkebek NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res 2007;51:899-911.)*

Phthalates/*Ftalati*		Metabolites/*Metaboliti*	
di-methyl-phthalate/*di-metil-ftalat*	DMP	mono-methyl-phthalate/*mono-metil-ftalat*	MMP
di-ethyl-phthalate/*di-etil-ftalat*	DEP	mono-ethyl-phthalate/*mono-etil-ftalat*	MEP
di-n-butyl phthalate/*di-n-butil-ftalat*	DBP	mono-butyl phthalate/*mono-butil-ftalat*	MBP
di-n-butil-phthalate/*di-n-butil-ftalat*	DBP	mono-butil-ftalat/*mono-izo-butil-ftalat*	MBP
di-iso-butyl phthalate/*di-izo-butil-ftalat*	DiBP	mono-iso-butyl phthalate/*mono-butil-benzil ftalat*	MiBP
butyl benzyl phthalate/*butil-benzil-ftalat*	BBzP	mono-butyl benzyl phthalate/*mono2-etil-heksil-ftalat*	MBzP
di-2-ethyl-hexyl phthalate *di-2-etil-heksilftalat*	DEHP	mono2-ethyl -hexyl phthalate *mono-2-etil-5 hidroksiheksil ftalat*	MEHP
		mono-2-ethyl-5 hydroxyhexyl phthalate *mono-2-etil-5 oksoheksil ftalat*	5OHMEHP
		mono-2-ethyl-5 oxohexyl phthalate *mono-2-etil-5 karboksipentil ftalat*	5oxoMEHP
		mono--2-ethyl-5 carboxy pentyl phthalate *mono-2-etil-5 karboksipentil ftalat*	5chMEHP
		mono-2-carboxy-hexyl-phthalate *mono-2-carboksi-heksil-ftalat*	2chMMHP
di-iso-nonyl phthalate/*di-izo-nonil-ftalat*		mono-iso-nonyl phthalate/*mono-izo-nonil-ftalat*	MiNP
		mono-hydroxy-iso-nonyl phthalatet *mono-hidroksi-izo-nonil-ftalat*	OH-MiNP
		mono-oxo-iso-nonyl phthalate *mono-okso-izo-nonil-ftalat*	oxo-MiNP
		mono-carboxy-iso-octyl phthalate *mono-karboksi-izo-oktil-ftalat*	cx-MiNP

of the seminal vesicles, prostate, and epididymis [18, 20]. According to contemporary literature, the stated syndrome is a consequence of reduced level of fetal testosterone, insulin-like growth factor-3 (IGF-3) and follicule stimulating hormone (FSH) [18, 21]. A negative correlation between levels in breast milk and free testosterone of babies was observed, while there was a positive correlation between mono-ethyl phthalate (MEP) and mono-butyl (MBP) with sex hormone bingind globuline (SHBG) and mono-metyl phthalate (MMP) and MEP and MBP with the ratio of lutenzing hormone (LH) and free testosterone [22].

Other effects of phthalates on health

Exposure to phthalates is significantly associated with the duration of pregnancy [2]. According to some studies, the chemical structure of DEHP and prostaglandin/thromboxane, interleukin-1 connects the phthalates with induction of intrauterine inflammatory processes as well as shortening of pregnancy [2, 23]. Some results suggested an association between levels of mono-(2-ethylhexyl) phthalate in preconceptional period and early pregnancy loss, while many authors pointed out the impact of phthalates on the low birth weight [9, 16, 18].

Recent research suggests a possible effect on neurocognitive development, as well as on the development of allergies, asthma, testicular carcinoma, hepatic and renal damages, insulin resistance and obesity, thyroid dysfunction [18].

An interesting fact is that exposure to a certain type of phthalates varies among different socioeconomic groups, which is probably the consequence of certain products whose use is significantly different among these groups [24].

Conclusion

Considering the widespread use of phthalates and exposure of large human population to phthalates in the environment, food or items for personal use their harmful impact on health need to be tested. Numerous experimental, epidemiological and observational studies of human population have suggested their most common side effects, but there are still many uncertainties. It is characteristic that detrimental effect is not only dose dependent. The duration of exposure is rather important: exposure to low doses of phthalates over a long period of time can lead to endocrine and metabolic disorders. Especially sensitive categories are the fetus and newborn, as well as pubertal

children. Endocrine disrupting chemicals have the epigenetic influence and these disorders can be manifested in the next generations. Further research aimed at timely recognition of adverse effects and adjusting the concentrations of the chemical compounds in products is needed in order to avoid their adverse effects on human health.

References

1. Frederiksen H, Skakkebek NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res 2007;51:899-911.

2. Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 2003;111:1783-5.

3. CDC's Fourth national report on human exposure to environmental chemicals, updated tables, february 2012; Available from: http://www.cdc.gov/exposurereport.

4. Commission directive 2004/93/EC of 21 September 2004 amending council directive 76/768/EEC for the purpose of adapting its Annexes II and III to technical progress. Brussels: European Parliament; 2004.

5. Heindel JJ, Powell CJ. phthalate ester effects on rat Sertoli cell function in vitro: effects of phthalate side chain and age og animal. Toxicol Appl Pharmacol. 1992;115:116-23.

6. Blount BC, Silva MJ, Caudill SP, Needham LL, et al. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 2000;108:979-82.

7. Koch HM, Bolt HM, Preuss R, Angerer J. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium labeled DEHP. Arch Toxicol 2005;79:367-76.

8. Silva MJ, Barr DB, Reidy JA, Kato K, Malek NA, Hodge CC, et al. Glucuronidation patterns of common urinary and serum monoester phthalates metabolites. Arch Toxicol 2003;77:561-7.

9. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr 2013;25:247-54.

10. Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, et al. Food packaging and bisphenol A and bis(2-ethylhexyl)phthalate exposure: findings from a dietary intervention. Environ Health Perspect 2011;119:914-20.

11. Hernandez-Diaz S, Mitchell AA, Kelly KE, Calafat AM, Hauser R. Medications as a potential source of exposure to phthalates in U.S. population. Environ Health Perspect 2009;117:185-9.

12. Wormuth M, Scheringer M, Vollenweider M, Hungerbuchler K. What are the sources of exposure to eight frequently used phtalic acid esters in Europeans? Risk Anal 2006;26:803-24.

13. Kato K, Silva M, Needham L, Calafat A. Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line SPE and isotope-dilution high performance liquid chromatography-tandem mass spectrometry. Anal Chem 2006;78:6651-5.

14. Silva M, Reidy J, Samandar E, Herbert A, Needham L, Calafat A. Detection of phthalate metabolites in human saliva. Arch Toxicol 2005;79:647-52.

15. Calafat AM, McKee RH. Integrating biomonitoring exposure data into the risk assasment process: phthalates (diethyl phthalate and di(2-ethylhexyl)phthalate) as a case study. Environ Health Perspect 2006;114:1783-9.

16. Braun JM. Smith KW, Williams PL. Variability of urine phthalate metabolite an bisphenol a concentrations before and during pregnancy. Environ Health Perspect 2012;120:739-45.

17. Hauser R, Meeker JD, Park S, Silva JM, Calafat AM. Temporal variability of urinary phthalate metabolit levels in men of reproductive age. Environ Health Perspect 2004;112:1734-40.

18. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res 2008;108:177-84.

19. Foster PM, Mylchreest E, Gaido KW, Sar M. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 2001;7:231-5.

20. Welsh M, Saunders PT, Fisken M, Scott HM, Hutchinson HM, Smith LB, et al. Identification in rats of a programming window for reproductive tract masculinisation, disruption of which leads to hypospadias and cryptorchism. J Clin Invest 2008;118:1478-90.

21. Sharpe RM, Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? Br Med J 2004;328:447-51.

22. Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damagaard IN, et al. Human breast milk contamination with phthalates alternations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect 2006;114:270-6.

23. Maroziene L, Grazuleviciene R. Maternal exposure to low-level air pollution and pregnancy outcomes: a population based study. Environ Health Perspect 2002;9:6.

24. Kobrosly RW, Parlett LE, Stahlhut RW, Barret ES, Swan SH. Socioeconomic factors and phthalate metabolite concentratins among United States women of reproductive age. Environ Res 2012;115:11-7.

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CASE REPORTS
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Case report
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APPLICATION OF FIBRIN RICH BLOCKS WITH CONCENTRATED GROWTH FACTORS IN PRE-IMPLANT AUGMENTATION PROCEDURES

UPOTREBA FIBRINSKIH BLOKOVA BOGATIH KONCENTROVANIM FAKTORIMA RASTA U PREIMPLANTOLOŠKIM AUGMENTACIONIM PROCEDURAMA

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Summary

Introduction. Growth factors are mediators regulating the key processes of tissue regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. In addition to the role they play in haemostasis and inflammatory processes, thrombocytes are of major importance in the reparation of mineralized and soft tissues. Application of fibrin rich blocks with concentrated growth factors is one of the latest approaches to guided bone regeneration and augmentation of lost bony structures of the alveolar ridge. **Case report.** This paper presents a case of a female patient who underwent reconstruction of the defect of residual alveolar ridge of the upper jaw by applying fibrin rich blocks with concentrated growth factors and subsequent placement of two titanium endosteal implants five months after wound healing. **Conclusion.** The loss of a single tooth or several teeth sometimes entails the augmentation of lost bony structures in order to provide optimal conditions for dental implant placement and subsequent prosthetic rehabilitation. A range of contemporary surgical procedures and a variety of dental materials for reconstruction of bony defects of the upper and lower jaws are available nowadays. The method described in this paper, i.e. the application of concentrated growth factors is one of the latest approaches which poses no risk of transmissible and allergic diseases and is at the same time cost effective.

Key words: Alveolar Bone Loss; Alveolar Ridge Augmentation; Intercellular Signaling Peptides and Proteins; Surgery, Oral; Female; Adult; Dental Implantation, Endosseous

Sažetak

Uvod. Faktori rasta su biološki medijatori koji regulišu ključne procese u reparaciji tkiva, uključujući ćelijsku proliferaciju, diferencijaciju, sintezu ekstracelulanog matriksa, hemotaksu i angiogenezu. Osim učešća u hemostazi i inflamaciji, trombociti imaju veliku ulogu u reparaciji mineralizovanih i mekih tkiva. Upotreba fibrinskih blokova bogatih koncentrovanim faktorima rasta predstavlja jednu od najsavremenijih metoda koja se koristi kod vođene koštane regeneracije pri nadoknadi izgubljenih koštanih struktura alveolarnog grebena viličnih kostiju. **Prikaz slučaja.** U radu je prikazan slučaj pacijentkinje kod koje je urađena rekonstrukcija defekata rezidualnog alveolarnog grebena gornje vilice fibrinskim blokovima bogatim koncentrovanim faktorima rasta, u koji su nakon perioda zarastanja od pet meseci ugrađena dva titanijumska endoosealna implantata. **Zaključak.** U slučajevima gubitka jednog ili više zuba, nekada je neophodna nadoknada izgubljenih koštanih struktura radi obezbeđivanja optimalnih uslova za ugradnju dentalnih implantata i protetsku rehabilitaciju koja sledi. Savremenim kliničarima na raspolaganju stoje brojne operativne procedure kao i veliki broj različitih materijala koji se koriste u rekonstrukciji koštanih defekata gornje i donje vilice. Prikazana metoda rada sa preparatima koncentrovanih faktora rasta spada u jednu od najsavremenijih, bez opasnosti od prenošenja transmisionih bolesti, pojave alergijskih reakcija. U ekonomskom smislu je u potpunosti isplativa.

Ključne reči: Gubitak alveolarne kosti; Nadoknada alveolarnog grebena; Faktori rasta; Oralna hirurgija; Žensko; Odrasli; Stomatološka endosealna implantacija

Introduction

In our everyday clinical practice, we face a relatively large number of patients presenting with a substantial deficit of the residual alveolar ridge due to the loss of single or several teeth who require implant-prosthetic treatment. In such situations, the augmentation of lost bony structures is indicated in order to provide optimal conditions for dental implant placement and subsequent prosthetic rehabilitation [1]. Nowadays, a whole range of modern surgical procedures and a variety of dental materials for reconstruction of bony defects of the upper and lower jaws and for the augmentation of lost structu-

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Abbreviations

CGF – concentrated growth factors
PRP – platelet rich plasma
PRGF – platelets rich in growth factor
PRF – platelet-rich fibrin
CBCT – cone beam computed tomography
GBR – guided bone regeneration

res of the residual alveolar ridge are available. The application of fibrin rich blocks with concentrated growth factors (CGF) is one of the latest approaches to guided bone regeneration (GBR). Fibrin rich blocks are applicable either alone or mixed/combined with any of synthetic bone grafts [1, 2].

According to numerous studies, growth factors are mainly located in blood plasma and thrombocytes. Growth factors are biological mediators regulating the major processes of tissue restoration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. In addition to the role they play in haemostasis and inflammatory processes, thrombocytes are of major importance in the reparation of mineralized and soft tissues [3].

The most important and most extensively investigated growth factors are platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) [4]. The first generation of these preparations, platelet rich plasma (PRP), has been well known for more than 15 years. PRP was first introduced into clinical practice by Marx in 1998 [5], whereas platelets rich in growth factor (PRGF) are the second-generation platelet concentrates. Platelet-rich fibrin (PRF) was first introduced by Choukroun in 2000 as the platelet concentrates of the third generation along with CGF introduced by Sacco in 2006. CGFs are characterized by higher density, larger amount of growth factors, higher viscosity and better adhesion capacity as compared to PRF. All preparations are produced from the patient's fresh venous blood [6].

The application of autologous fibrin rich blocks has no adverse effects; it is considered a safe and simple procedure for the surgeon. At the same time, it is highly effective and economically feasible for the patient.

Case report

A 28-year-old female patient came to the Department of Oral Surgery and Implantology of the Dental Clinic of Vojvodina because of the loss of tooth 22 and pain in the region of tooth 23. The clinical examination revealed fresh extraction wound after the last extraction of tooth 22 and intraoral fistula in the root tip area of the tooth 23. The patient was referred to cone beam computed tomography (CBCT) scan. The CBCT scan revealed a radicular cyst on tooth 23 associated with massive destruction of the surrounding bony tissue (**Figure 1**).

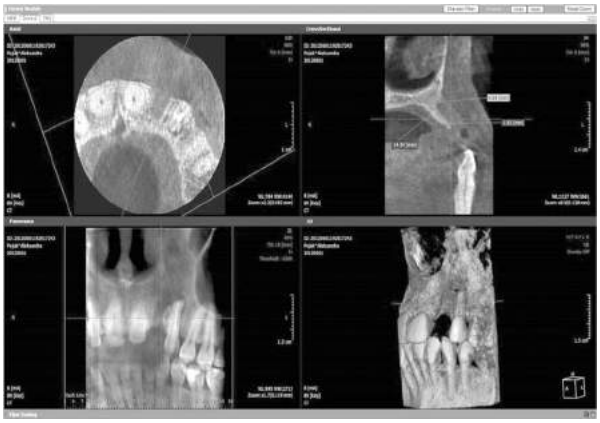

Figure 1. Preoperative CBCT scan
Slika 1. *Preoperativni CBCT snimak*

After completing the diagnostic procedure and consulting the specialist in dental prosthetics, the therapy options were considered. The definitive therapeutic strategy was established, encompassing three stages. Stage 1 included the extraction of tooth 23, cyst curettage and the reconstruction of bony defects in regions of teeth 22 and 23 by applying CGF blocks. The second stage, after bone healing, included the placement of two endoosseous titanium implants at the position of teeth 22 and 23. The third stage, subsequent to the successfully completed osseointegration, included the placement of two implant-supported metal-ceramic crowns. After obtaining the patient's written consent, the suggested therapy plan was carried out. After full-thickness mucoperiosteal flap had been lifted, the extraction of tooth 23 along with complete curettage of the cystic process and surrounding pathologically altered tissue was performed under local anaesthesia. The resulting bony defects were entirely filled out with CGF blocks previously prepared from the patient's venous blood and the flap was sutured back in place with synthetic non-absorbable monofilament suture (**figures 2 and 3**).

Five months after surgery, the control CBCT scan revealed the complete regeneration and reparation of the bone tissue and satisfactory dimensions of the residual alveolar ridge, that being the prerequisite for the placement of endoosseous implants of 3.3 mm in diameter and 12 mm in length. (**figures 4 and 5**). After implantation, the entire surgical region was covered with pressed CGF barrier membrane and the flap was put back in place and sutured with synthetic non-absorbable monofilament suture in order to minimize the accumulation of soft deposits and to alleviate potential tissue reactions [7, 8].

Four months after implantation procedure, the patient underwent prosthetic restoration procedure at the Department of Dental Prosthetics. The procedure included the placement of two implant-supported metal-ceramic crowns (**Figure 6**).

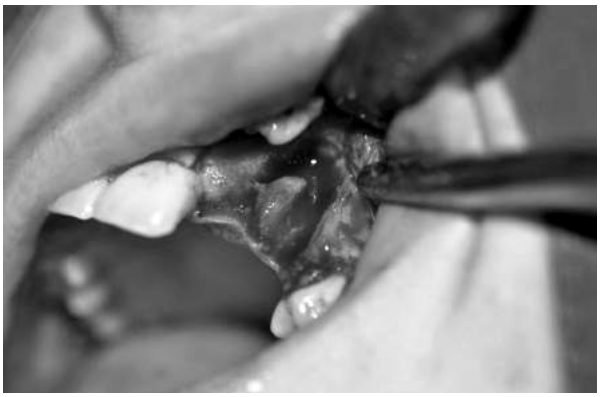

Figure 2. Intraoperative bony defect
Slika 2. *Intraoperativni koštani defekt*

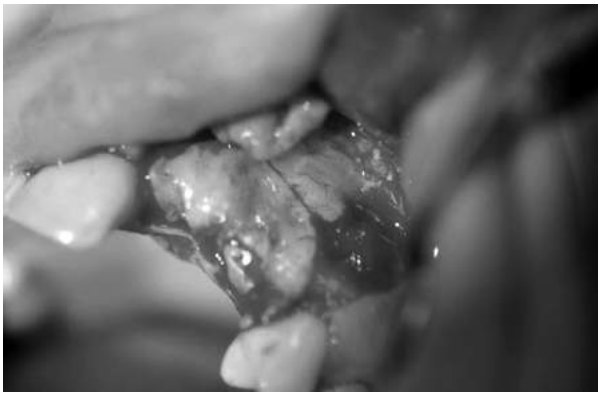

Figure 3. Inserted CGF blocks
Slika 3. *Postavljeni CGF preparati*

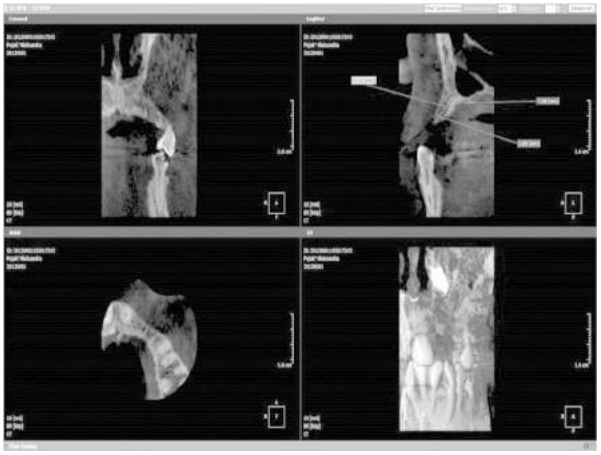

Figure 4. Control CBCT scan
Slika 4. *Kontrolni CBCT snimak*

Discussion

Application of CGF blocks is one of the most recent approaches to practical application of tissue engineering methods in oral surgery and implantology [4]. This is of particular importance in implantology, having in mind the growing need for augmentation procedures in cases of unfavourable anatomic conditions (horizontal and vertical augmentation, sinus-lift, etc.) [2].

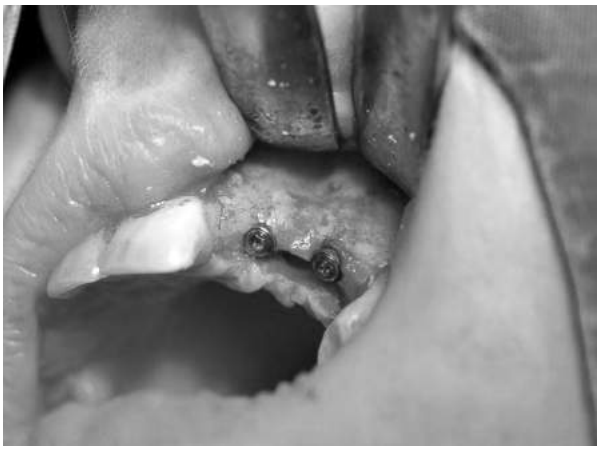

Figure 5. Inserted endosteal implants
Slika 5. *Postavljeni endoosealni implantati*

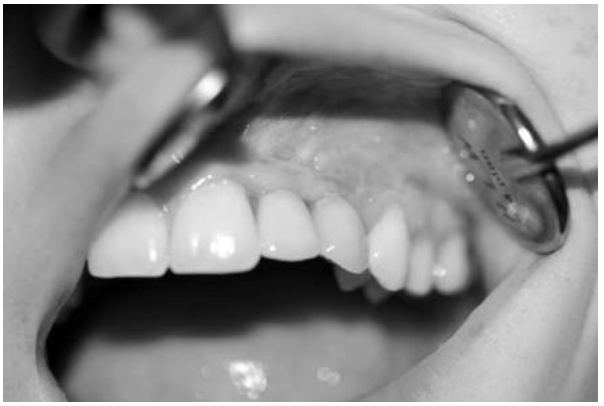

Figure 6. Definitive appearance after treatment
Slika 6. *Definitivni izgled nakon terapije*

The comparison of protocols for CGF block preparation revealed different utilization rates of collected blood, being 10% in PRP and PRGF and 30–40% in PRF and CGF. Numerous authors have reported positive results of the application of CGF in the augmentation of bone architecture of the residual alveolar ridge and establishment of adequate anatomical conditions for a successful implant-prosthetic rehabilitation of toothless patients. Blocks with CGF enhance the processes of bony structure reparation and regeneration thus shortening the healing time from the initial surgical procedure, i.e. alveolar ridge augmentation to the moment of placing the endoosseous implant into the augmented region. As aforementioned, CGF blocks are applicable alone or combined with any of synthetic bone grafts. However, novel trends in GBR give preference to natural graft material, i.e. autotransplants. In a broader sense, CGF blocks could be considered natural graft material [1, 2, 6].

Conclusion

In our everyday clinical practice, we face a relatively large number of patients indicated for implant-prosthetic treatment. The loss of a single tooth or several teeth results in substantial deficit of the residual alveolar ridge in such patients. Such situations require the augmentation of lost bony structures in order to provide optimal conditions for dental implant placement and subsequent prosthetic rehabilitation. Oral surgeons have the whole range of modern surgical procedures and a variety of dental materials for reconstruction of bony defects of the upper and lower jaws at their disposal. The method presented in this paper, i.e. the application of concentrated growth factors is one of the latest approaches, which carries no risk of either transmissible or allergic diseases, and is at the same time highly effective and economically feasible.

References

1. Mirković S, Đurđević Mirković T, Puškar T. Application of concetrated growth factors in reconstruction of bone defects after removal of large jaw cyst: report of two cases. Vojnosanit Pregl 2014 in press.

2. Mirković S, Đurđević Mirković T, Petrović L, Božić D. The use of concentrate growth factors in gyded bone regeneration after lateral sinus lift procedure: case report. HealthMED 2013;7(2):700-4.

3. Plachokova AS, Nikolidakis D, Mulder J, Jansen JA, Creugers NH. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clin Oral Implant Res 2008;19(6):539-45.

4. Lazić Z, Bubalo M, Petković-Ćurčin A, Duka M, Mihajlović B. Terapijska primena plazme bogate trombocitima u oralnoj hirurgiji. Vojnosanit Pregl 2009;66(10):821-5.

5. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(6):638-46.

6. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C. Platelet-rich fibrin (PRF): a second generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):56-60.

7. Mirković S, Džambas LJ, Selaković S. Uticaj različitih materijala za šivenje na intenzitet tkivne reakcije usne šupljine. Zb Matice Srpske Prir Nauke 2008;115:91-9.

8. Mirković S, Đurđević Mirković T, Bajkin B, Šarčev I. Izbor hirurškog šavnog materijala prilikom suturiranja u usnoj duplji: klinička studija. Med Pregl 2010;63(7-8):497-501.

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Case report
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SEVERE HYPERKALEMIA INDUCED BY PROPRANOLOL

TEŠKA HIPERKALIJEMIJA IZAZVANA PROPRANOLOLOM

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Summary

Introduction. Hyperkalemia secondary to beta-adrenergic receptor blockade occurs in 1-5% of patients and is likely to develop with non-cardio-selective beta-blockers. **Case Report.** We have described hyperkalemia in a patient with angina pectoris receiving propranolol, clinically manifested as weakness, tightness behind the sternum and numbness in the limbs. Laboratory tests showed hyperkalemia (6.6 mmol/L), peaked T wave and a corrected QT interval of 510 ms. After discontinuation of propranolol, decline in potassium level, normalisation of electrocardiographic changes and clinical improvement were achieved. Causal relationship of drug related hyperkalemia has been confirmed as probable/likely according to Naranjo Adverse Drug Reaction Probability Score of 7 and the World Health Organization Uppsala Monitoring Centre Probability Scale. **Conclusion.** Hyperkalemia can be unpredictable and life-threatening complication of propranolol or a non-selective adrenergic beta blocker treatment, and requires timely identification of cause and implementation of therapeutic measures.

Key words: Hyperkalemia; Propranolol; Electrocardiography; Drug-Related Side Effects and Adverse Reactions; Signs and Symptoms; Female; Middle Aged; Angina Pectoris; Risk Factors

Sažetak

Uvod. Hiperkalijemija uzrokovana blokadom beta adrenergičkih receptora javlja se kod 1−5% pacijenata, a najčešće je izazvana neselektivnim beta blokatorima. **Prikaz slučaja**. Prikazali smo pacijentkinju s hiperkalijemijom i kliničkim manifestacijama u vidu slabosti, bolova iza grudne kosti i trnjenja u ekstremitetima, koja je uzimala propranolol zbog angine pektoris. Laboratorijske analize pokazale su hiperkalijemiju (6,6 mmol/l), visoke T-talase i korigovani QT interval od 510 ms. Nakon prekida uzimanja propranolola, dolazi do pada nivoa kalijuma, normalizacije elektrokardiografskih promena i poboljšanja kliničkog nalaza. Uzročna povezanost između leka i hiperkalijemije klasifikovana je kao verovatna prema Naranjo skali za procenu verovatnoće skorom 7 i *World Health Organization Uppsala Monitoring Centre* skalom verovatnoće. **Zaključak.** Hiperkalijemija može biti nepredvidiva i po život opasna komplikacija primene propranolola ili drugih neselektivnih beta blokatora i zahteva pravovremenu identifikaciju i sprovođenje terapijskih mera.

Klučne reči: Hiperkalijemija; Propranolol; Elektrokardiogram; Nus efekti i neželjene reakcije izazvane lekovima; Znaci i simptomi; Žensko; Srednje godine; Angina pectoris; Faktori rizika

Introduction

Potassium is the principal intracellular cation, and maintenance of its distribution between the intracellular and the extracellular compartments relies on several homeostatic mechanisms. When these mechanisms are perturbed, hypokalemia or hyperkalemia may occur [1]. Severe hyperkalemia increases the risk of fatal dysrhythmias and therefore early recognition of its electrocardiographic (ECG) manifestations is clinically very important. The ECG changes associated with high potassium level may include peaked T waves, prolongation of the PR interval, and QRS widening followed by loss of atrial activity, ventricular fibrillation, and asystole. The pathological ECG changes of hyperkalemia are proportional to the serum potassium level, and can be detected when its level is greater than 6.0 mmol/L [1].

Case report

A female patient, aged 50, was referred to an internal medical examination as she was experiencing a feeling of general weakness, tightness behind the sternum and numbness in the limbs, the symptoms that had been occurring increasingly over the previous month. The medical history showed that a week prior to the onset of the symptoms, the patient had been diagnosed with angina pectoris and therefore she had been receiving propranolol a 40 mg per day, divided into two doses. Additionally, the patient was fasting for two weeks over the previous month due to a dental intervention. The medical history also showed the presence of comorbid hypothyreosis and osteoporosis, treated with 100 μg levothyroxine daily and calcium supplement. The clinical examination established the general condition to be relatively good with arterial blood pressure 120/70 mmHg and normal auscultatory find-

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Abbreviations
ECG – electrocardiographic

ings. The laboratory test results showed hyperkalemia (6.6 mmol/L), while the other – blood glucose, electrolyte, serum creatinine and uric acid levels, as well as hormonal status of thyroid gland were within the reference range (**Table 1**). However, her ECG showed the sinus rhythm having a heart rate of 55 beats per minute, a PQ interval of 0,16" and tall, peaked T waves in the precordial leads, with a corrected QT interval (QTc) of 510 ms **(Figure 1).** A repeated electrolyte analysis the day after showed the persistence of hyperkalemia (6.5 mmol/l) and pathological ECG. As propranolol was suspected to be the possible cause of the increase in potassium level and slow heart rate as well, the dosage was reduced to 20 mg per day. After three days, the follow-up laboratory findings revealed a slight gradual decline in potassium level to 6.2 mmol/L. Propranolol was discontinued and intensive antihyperkalemia therapy was initiated as a single intravenous bolus of 1 mg/kg furosemide followed by 1 mg/kg per day intravenous furosemide. This therapy led to normalization of the patient's serum potassium levels two days after. Propranolol was replaced by a selective beta blocker bisoprolol at a dose of 2.5 mg per day. At follow-up, on the second and seventh day as well as two weeks after bisoprolol had been initiated, potassium level remained stable within the normal range (4.5-5.6 mmol/L) (**Table 1**), ECG changes normalized and the patient denied experiencing the aforementioned symptoms.

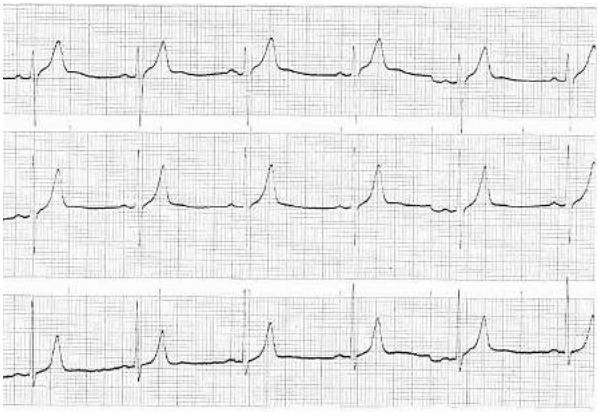

Figure 1. ECG in the precordial leads (V4-V6)
A sinus rhythm (55 beats/min), with the absence of P waves, a prolonged QT interval and peaked T waves is shown.
Slika 1. *Elektrokardiogram u prekordijalnim odvodima (V4−V6)*
Prikazan je sinusni ritam (frekvencija 55/min), sa odsustvom P-talasa, prolongiranim QT intervalom i šiljatim T-talasima.

Discussion

This report presents a case of hyperkalemia which was most likely caused by propranolol. The causal relationship between the serum potassium level and the propranolol treatment is supported by the close temporal relationship between the drug administration and hyperkalemia, positive *Dechallenge*, e.g. the potassium level normalization following the propranolol discontinuation, and the absence of interaction with the concomitant drugs. However, the reduced food intake can be considered as an additional risk factor for potassium disposition disorder. Fasting-induced hyperkalemia in patients with end-stage renal disease is a well-known phenomenon, caused by reduced insulin secretion and beta-adrenergic receptor sensitivity as well [2]. Taking into consideration clinical examination, laboratory test, beta blocker pharmacology and medical history, the causal relationship of propranolol related hyperkalemia has been confirmed as probable/likely according to World Health Organization Collaborating Centre for International Drug Monitoring, the Uppsala Monitoring Centre (WHO–UMC) with score of 7 according to Naranjo Adverse Drug Reaction Probability Scale [3, 4].

Potassium is a main intracellular cation in human body. Nearly 98% of potassium is intracellular, and the concentration gradient is maintained by the Na-K-ATP pump activity. Small changes in the extracellular potassium level can have an effect on the function of the vascular and neuromuscular systems. Hyperkalemia is often a life-threatening clinical condition due to the risk of potentially fatal arrhythmias [1]. It is often associated with certain conditions and diseases, such as intense exercise, status epilepticus, trauma, rhabdomyolysis, starvation, renal failure and or it can be drug induced. The mechanism for the development of hyperkalemia is explained by either an excess release of potassium from skeletal muscles or its reduced uptake by those muscles [5–7].

Drugs that most commonly cause hyperkalemia are potassium-sparing diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, adrenergic beta blockers, potassium supplements, non-steroid anti-inflammatory drugs, cyclosporine A, digoxin intoxication and renin antagonist. Adrenergic beta-blocker-induced hyperkalemia occurs in 1-5% of treated patients and is more common in patients taking non-selective beta-blockers, such as propranolol, carvedilol, and labetalol, versus those taking cardio-selective beta blockers [2, 5, 7]. Severe hyperkalemia induced by propranolol has been reported in patients with heart failure as well as in children receiving this drug in the treatment of infantile haemangioma [8, 9].

Given that non-selective adrenergic beta blockers have an effect on beta 2 receptors and affect Na and K homeostasis, there are two proposed mechanisms assumed to be responsible for the development of hyperkalemia such as renin-angiotensin-aldosterone system blockade and decrease

Table 1. Laboratory findings of propranolol-induced hyperkalemia in a 50-year old female patient treated with daily dose of 40 mg propranolol, 100 μg levothyroxine daily and calcium supplement
Tabela 1. *Laboratorijski nalazi hiperkalijemije indukovane propranololom kod pacijentkinje starosti 50 godina tretirane dnevnom dozom propranolola 40 mg, levotiroksina 100 μg i dodatkom kalcijuma*

Test *Testovi*	Reference range *Referentni opseg*	During propranolol treatment *Tokom primene propranolola*	After discontinuation of propranolol *Nakon prekida primene propranolola*
RBC count/*Eritrociti*	4−5 x 10^{12} /l	4,53 x 10^{12}/l	4,42 x 10^{12}/l
Hemoglobin/*Hemoglobin*	110−160 g/l	118g/l	118g/l
Hematocrit/*Hematokrit*	0,37−0,47	0,40	0,41
Platelet count/*Trombociti*	150−450 x 10^9/l	168 x 10^9/l	172 x $10^{9/l}$
WBC count/*Leukociti*	4−10 x 10^9/l	7,21 x 10^9/l	6,54 x 10^9/l
Urea/*Urea*	2−8 mmol/l	3,1 mmol/l	3,5 mmol/l
Creatinine/*Kreatinin*	53−106 μmol/l	64 μmol/l	74 μmol/l
Sodium/*Natrij*	130−147 mmol/l	136 mmol/l	140 mmol/l
Potassium/*Kalij*	3,2−5,2 mmol/l	6,6 mmol/l	4,8 mmol/l
Chloride/*Hloridi*	95−105 mmol/l	99 mol/l	99 mmol/l
pH	7,35−7,45	7,36	7,40
pCO_2	4,66−5,98 kPa	4,87 kPa	5,00 kPa
pO_2	9,98−13,3 kPa	10,8 kPa	10,1kPa
sO_2	95−98 %	95%	96%
ABE	-1 ± 2,3 mmol/l	1 mmol/l	1,8 mmol/l
HCO_3	22−26 mmol/l	25 mmol/l	26mmol/l
Glucose/*Šećer u krvi*	3−6,1 mmol/l	4,0 mmol/l	4,2 mmol/l
Total protein *Ukupni proteini (serum)*	62−75 g/l	63 g/l	65 g/l
Albumin/*Albumini*	35−53 g/l	40 g/l	41g/l
AST	10−40 IU/l	23 IU/l	23 IU/l
ALT	7−56 IU/l	25 IU/l	30 IU/l
TSH	0,27−4,2 mIU/l	2,34 mIU/l	2,35 mIU/l
GGT	30−120 IU/l	65 IU/l	60 IU/l
LDH	90−340 IU/l	240 IU/l	268 IU/l

RBC – red blood cells/*crvena krvna zrnca*, WBC – white blood cells/*bela krvna zrnca*, ABE – actual base excess/*stvarni bazni eksces*; AST – Aspartate transaminase/*asparat amino trasferaza;* ALT – Alanine transaminase/*alanin amino transferaza;* TSH – Thyroid-stimulating hormone/*tiro-stimulišući hormon*; GGT – Gamma glutamyltransferase/*gama glutamil transferaza*; LDH – Lactate dehydrogenase/*laktat dehidrogenaza*; IU – international unit/*internacionalne jedinice*

potassium uptake from the extracellular space into the cell. It is well known that adrenergic beta blockers inhibit catecholamine-induced stimulation of renin release, and lead to reduced aldosterone production and potassium retention. In addition, blockade of beta 2 receptors leads to inhibition of the cell membrane Na-K-ATP pump (similarly to digoxin), which is responsible for cellular Na and K homeostasis [2, 5, 10].

Conclusion

Hyperkalemia can be unpredictable and life-threatening complication of propranolol or a non-selective adrenergic beta blocker treatment. Drugs that can disrupt the intracellular/extracellular potassium balance or lead to an impaired regulation of potassium excretion should be taken into account. Therefore, early recognition of these symptoms, close monitoring of patient's with electrocardiographic and electrolyte status, discontinuation of a suspected drug and specific therapeutic measures are required.

References

1. Evans KJ, Greenberg A. Hyperkalemia: a review. J Intens Care Med 2005;20:272-90.

2. Nowicki M, Miszczak-Kuban J. Nonselective beta-adrenergic blockade augments fasting hyperkalemia in hemodialysis patients. Nephron 2002;91:222-7.

3. The use of the WHO–UMC system for standardized case causality assessment. Accessed from: http://www.WHO-UMC.org/graphics/4409.pdf [Last accessed on 2014 Jan 12].

4. Parida S. Clinical causality assessment for adverse drug reactions. Indian J Anaesth 2013;57:325-6.

5. Clausen T. Hormonal and pharmacological modification of plasma potassium homeostasis. Fundam Clin Pharmacol 2010;24:595-605.

6. Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. New Engl J Med 2009;36:62-72.

7. Liamis G, Milionis H, Elisaf M. Blood pressure drug therapy and electrolyte disturbances. Int J Clin Pract 2008;62:1572-80.

8. Maia ER, Villacorta H, Subietta CG, Munhoz C, Romeo Filho LJ, Mesquita ET. Use of propranolol in heart failure patients: safety, tolerability, and effects on left ventricular function. Rev Port Cardiol 2001;20:383-99.

9. Pavlakovic H, Kietz S, Lauerer P, Zutt M, Lakomek M. Hyperkalemia complicating propranolol treatment of an infantile hemangioma. Pediatrics 2010;126:1589-93.

10. Kokot F, Hyla-Klekot L. Drug-induced abnormalities of potassium metabolism. Pol Arch Med Wew 2008;118:431-3.

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Case report
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RETINAL HEMORRHAGES AS ONE OF COMPLICATIONS OF OPTIC DISC DRUSEN DURING PREGNANCY

RETINALNA HEMORAGIJA KAO KOMPLIKACIJA DRUZA OPTIČKOG DISKA U TRUDNOĆI

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Summary

Introduction. Drusen of the optic nerve head are relatively benign and asymptomatic. They represent retinal hyaline corpuscles resulting from impaired axoplasmic transport of the retinal ganglion cells of optic nerve in front of the lamina cribrosa. They are usually detected accidentally, during a routine ophthalmologic examination. Most patients with optic disc drusen are not aware of the deterioration of their eyesight because of the slow progression of visual field defects. Damage in visual acuity due to optic disc drusen is rare. **Case Report.** A 27-year-old female patient in the sixth month of pregnancy visited an ophthalmologist because of a visual impairment described as the appearance of mist and shadows over her right eye. When first examined, her visual acuity in both eyes was 20/20. The retinal hemorrhages framing the bottom half of the optic nerve were seen. Complete laboratory and clinical testing as well as specific ophthalmic examinations (photofundus, computerized visual field, optical coherence tomography, and ultrasound) were performed to exclude systemic causes and they presented no risk for the pregnancy. Echosonographic examination confirmed the presence of bilateral optic nerve head drusen. **Conclusion.** Hemodynamic changes during pregnancy are possible factors for the development of optical disc and retinal hemorrhages. Since treatment of optic disc drusen is limited, recognition of optic nerve drusen as a cause of hemorrhage during pregnancy prevents unnecessary diagnostic and therapeutic interventions.

Key words: Retinal Hemorrhage; Pregnancy; Optic Disk Drusen; Female; Adult; Early Diagnosis; Pregnancy Complications, Hematologic

Sažetak

Uvod. Druze optičkog diska su relativno benigna i asimptomatska pojava. Predstavljaju retinalna hijalina telašca nastala kao produkt narušenog aksoplazmatskog transporta retinalnih ganglijskih ćelija u vidnom živcu ispred *lamine cribrosae*. Otkrivaju se najčešće slučajno pri rutinskom oftalmološkom pregledu. Češće su kod žena i belaca. Većina pacijenata sa druzama optičkog diska nije svesna pogoršanja svog vidnog polja zbog lagane progresije. Oštećenje oštrine vida zbog druza optičkog diska je retko. **Prikaz slučaja.** Pacijentkinja starosti 27 godina, u šestom mesecu trudnoće javila se oftalmologu zbog subjektivnih smetnji u vidu magle i osećaja senke pred desnim okom. Pri prvom pregledu vidna oštrina oba oka bila je 1,0. Viđene su preretinalne i retinalne hemoragije koje uokviruju donju polovinu papile vidnog živca. Kompletno laboratorijsko i kliničko ispitivanje, kao i specifični oftalmološki pregledi (fotofundus, kompjuterizovano vidno polje, optička koherentna tomografija, ultrazvučni pregled) sprovedeni su da bi se isključili sistemski uzroci i iznela trudnoća bez rizika. Ehosonografski pregled je potvrdio obostrano prisustvo druza vidnog živca. **Zaključak.** Hemodinamičke promene u trudnoći su mogući faktor nastanka hemoragija optičkog diska i retine. Znajući da je tretman druza optičkog diska ograničen, prepoznavanje druza vidnog živca kao uzroka hemoragija u trudnoći sprečava nepotrebne dijagnostičke i terapijske intervencije.

Ključne reči: Retinalna hemoragija; Trudnoća; Druza optičkog diska; Žensko; Odrasli; Rana dijagnoza; Hematološke komplikacije u trudnoći

Introduction

Drusen of the optic nerve head are relatively benign and asymptomatic. They represent retinal hyaline corpuscles resulting from impaired axoplasmic transport of retinal ganglion cells of optic nerve in front of the lamina cribrosa [1, 2]. They are usually detected accidentally, during a routine ophthalmologic examination.

They are more frequent in women and Caucasians [3]. The prevalence of optic disc drusen (ODD) is between 3.4 and 24 per 1000 population [4–6].

They can cause peripheral visual field defects in 71% to 75% of the eyes. Clinical findings do not tend to deteriorate in most patients. Many theories explain the changes in the visual field, such as the direct compression on the axons of ganglion cells, ischemia of the optic nerve, a small scleral canal and impaired axonal transport [7]. The most common defects in the visual field are scotomas, particularly in the lower nasal quadrant, the extension of the blind spots and concentric narrowing of the visual field [8, 9]. However, these incidents are not correlated with the position of ODD in the optic

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Abbreviations

ODD	– optic disc drusen
IgM	– immunoglobulin M
IgG	– immunoglobulin G
HSV1	– herpes simplex virus 1
OCT	– optical coherence tomography
RNFL	– retinal nerve fiber layer

nerve head [9–12]. Large defects in the visual field and a reduction of central visual acuity are rare [10]. The only problems that patients notice are arcuate scotomas as the major cause of reduced vision [10–12]. Most patients with drusen are not aware of the deterioration of their sight because the slow progression of visual field defects.

In addition, ODD may cause anterior ischemic neuropathy, central retinal vein occlusion, repeated episodes of transient vision loss, optic nerve atrophy, venous occlusion, juxtapapillary choroidal neovascular membrane formation leading to subretinal hemorrhage and other complications [4, 7, 9, 11]. Unlike superficial drusen, the deep ones closer to lamina cribrisa are often associated with vascular changes on the optic nerve head because of greater compressive effect [9, 10].

Case Report

A 27-year-old female patient in the sixth month of pregnancy visited an ophthalmologist because of a visual impairment described as the appearance of mist and shadows over her right eye. When first examined, the visual acuity in both eyes was 20/20 according to Snellen charts, and the intraocular pressure was 17 mmHg bilaterally. Clinical examination on the slit lamp was uneventful. The fundus examination of the right eye revealed the swollen optic nerve head up to 1 diopter, the blood partly covered the lower half of the optic disc circular in shape, the size being of about 1.5 of papilla diameter, partly in layers of the retina and partly in preretinal parts. The veins were fuller. The rest of the retina was neat. The fundus examination of the left eye revealed a discretely swollen optic nerve head up to 0.5 diopter. Other findings were within the reference range (**Figure 1**).

The differential diagnosis included thrombosis of the central retinal vein branch or papillophlebitis. The patient was asked to undergo testing of complete blood count and phospholipid status, screening of coagulation factors, computerized visual field, optical coherence tomography of fundus, and check-up by the neurologist with nuclear magnetic resonance of endocranium. Fundus fluorescein angiography, which could have provided a definitive diagnosis, was contraindicated because of her pregnancy.

The results of blood biochemical analysis were within reference range, except for total cholesterol, which was 6.22 mmol/l (3.90 to 5.20). Virological tests (immunoglobulin M-IgM and immunoglobulin G -IgG for Coxsackievirus, Adenovirus, herpes simplex virus 1- HSV1, varicella-zoster virus - VZV) were negative, except for IgG, which was positive for adenovirus and HSV 1. Immunological features of the different antibodies were negative (anti - mitochondrial, anti-cardiolipin, anti-phospholipid IgG and IgM, anti - β2 - glycoprotein 1). Vascular and coagulation profiles and lupus anticoagulants showed no pathological significance. The results of color Doppler sonography of blood vessels of the lower extremities as well as magnetic resonance imaging of endocranium were within the physiological findings. Neurological examination was normal. Therefore, there were no systemic causes of retinal hemorrhage in the right eye, and both the mother and fetus were protected from additional systemic complications except for uncertain outcome regarding the right eye vision because of unclear etiology.

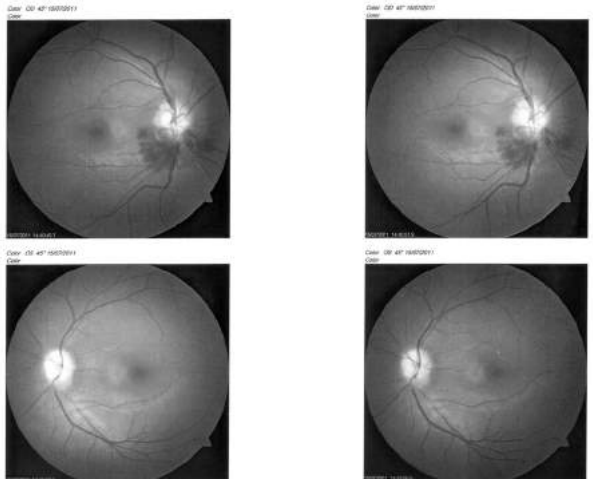

Figure 1. Photofundus of the right eye with retinal hemorrhage in the lower half and the left eye with normal features
Slika 1. *Fotofundus desnog oka sa retinalnom hemoragijom u donjoj polovini i levog oka sa normalnim nalazom*

Computerized visual field (Optopol, Sp.z.o.o., Zawiercie, Poland; glaucoma, fast threshold) was done on the day of first examination and revealed bilateral visual field defects, although the patient was not aware of visual loss in her left eye. The Bjerrum relative scotoma in the formation was seen as well as Rene's nasal step, which corresponded to the localization of retinal hemorrhages in her right eye. The visual field of the left eye showed the decrease of sensitivity of the retina, pericecal scotoma, and absolute and relative scotomas in the nasal Rene's zone (**Figure 2**).

Optical coherence tomography (OCT; Cirrus HD-OCT, Zeiss, Meditec, CA; retinal nerve fiber layer (RNFL) and optic nerve head test) showed an asymmetry in findings of the right and left eye, with the preservation of RNFL thickness, the neuroretinal rim thickened on the right eye due to extravasation and accumulation of fluid (Right eye Avg. RNFL Thick. 198 μm, Left eye 81 μm) (**Figure 3**).

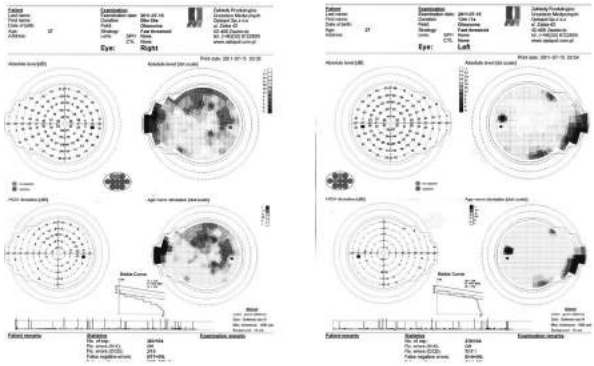

Figure 2. Computerized visual field of the right and left eye with changes
Slika 2. *Kompjuterizovano vidno polje desnog i levog oka sa ispadima*

After three weeks of therapy with anticoagulants (0.3 ml nadroparin calcium 9,500 anti- Xa IU/ml daily) and antioxidants (500 mg vitamin C daily), hemorrhages resolved spontaneously. Visual acuity was 20/20 in both eyes, the intraocular pressure 15 mmHg. A new fundus image was done, which showed drusen of the optic nerve head, which were no longer masked by hemorrhages. The ultrasound examination of both eyes (B scan; Ultrasound A/B Scanner, UD- 6000, Tomey Corp. USA) revealed a prominent, highly reflecting signal to the optic nerve bilaterally, confirming the diagnosis of drusen bilaterally (**Figure 4).**

Further monitoring of the patient after delivery, i.e. 10 months after the bleeding episode, included specific ophthalmologic examinations. The repeated OCT indicated the bilateral neuroretinal rim thickening and thinning of the peripapillary RNFL. Peripapillary RNFL of the right eye (Average RNFL Thickness 51 μm) was thinner in the superior (71 μm), inferior (52 μm) and temporal (34 μm) and nasal quadrant (48 μm). RNFL of the left eye (Average RNFL Thickness 82 μm) was thinner in the superior (66 μm) and nasal quadrant (49 μm). OCT showed macular thinning of the retinal nerve fibers in the superior, nasal and inferior quadrants of the right eye, while the contours of the fovea were bilaterally preserved and the central thickness of the fovea was normal (211 μm in the right eye and 216 μm in the left eye) (**Figure 5**).

Static perimetry (Humphrey Visual Field Analyzer, HFA, SAD; Threshold test C 30-2) revealed defects in the visual field of the right eye, blind spot enlargement, absolute central scotoma, absolute and relative paracentral scotoma (MD -9.08 dB, PSD 8.09 dB). The left eye findings indicated relative and absolute paracentral and nasal scotoma in the ”pattern” deviation (MD -2.10 dB, PSD 3.20 dB) (**Figure 6**). Computerized visual field and optical coherence tomography showed permanent damage of the retinal nerve fibers due to bleeding and compression.

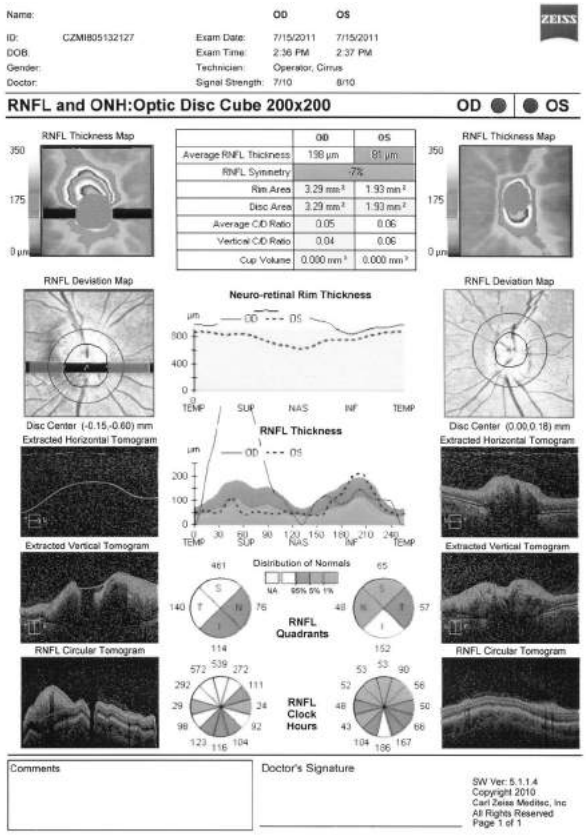

Figure 3. OCT findings of both eyes
Slika 3. *OCT nalaz oba oka*

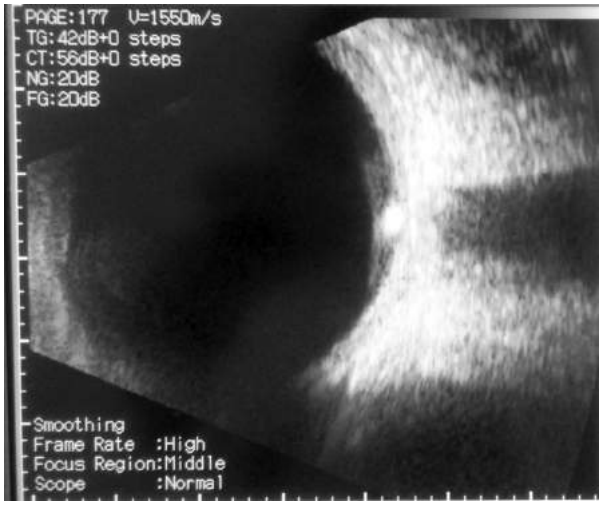

Figure 4. B scan of the right eye
Slika 4. *B sken desnog oka*

Discussion

Optic disk drusen are usually benign phenomena with well-preserved visual acuity. Patients often fail to perceive them since the central visual field loss is rare and it can occur due to hemorrhages of the optic disc, peripapilar retinal or vitreous hemor-

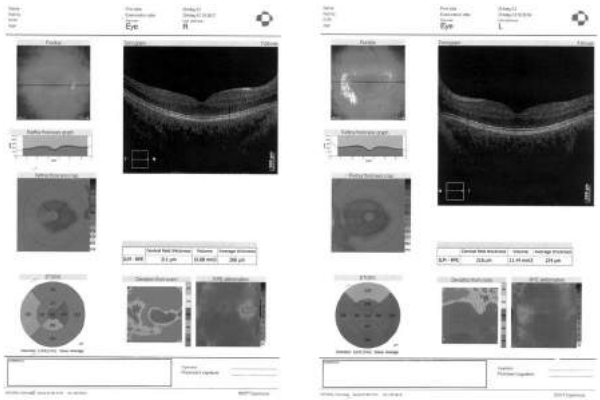

Figure 5. OCT of the macula in the right and left eye
Slika 5. *OCT makule desnog i levog oka*

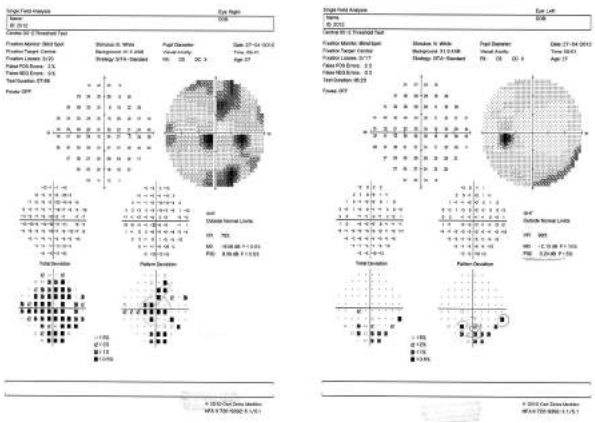

Figure 6. Visual fields of the right and left eye (Humphrey Visual Field Analyzer, Threshold test C 30-2)
Slika 6. *Vidno polje desnog i levog oka (Humphrey Visual Field Analyzer, Threshold test C 30-2)*

rhages, or ischemia which affects the retina or the optic nerve [2–7, 11, 12]. The diagnosis can be made only by ophthalmoscopy, although numerous additional differential diagnostic tests are available. Patients with optic disc drusen should be regularly monitored for possible complications because drusen may vary in their appearance and position throughout the life of the patient, from those deeply immersed in the optic nerve to the ones placed on the surface of the nerve [12–14].

The prevalence of retinal hemorrhages in patients with ODD is from 2% to 10% [15–18]. Sanders et al. distinguish four types of bleeding in the retina in relation to ODD: 1) small, transient, asymptomatic splinter hemorrhages on the head of the optical disc; 2) bleeding from the head of the optic nerve extending into the vitreous body and causing transient symptomatic defects in the visual field; 3) deep papillary hemorrhage, and 4) deep peripapillary hemorrhage that can infect the region of the macula, accompanied by severe visual impairment and permanent defects in the visual field [16, 19].

In most cases, retinal hemorrhages are detected accidentally, without the deterioration of visual acuity and no subretinal neovascularization, because the involvement of the macula and vision impairment are less frequent [17, 18, 20].

The pathological mechanism of bleeding in patients with ODD is not sufficiently understood; it may be due to erosion of the vessel wall because of the expansion of disk drusen or a change of the drusen position, the blood congestion or ischemia [18–21]. In addition, ischemic effect of drusen enlargement can stimulate proliferation of blood vessels at the border of the optic disc between the Bruch membrane and the pigment epithelium, whose rupture can produce profound peripapillary hemorrhages. A retinal hemorrhage described in a nine-year-old boy with ODD during a tennis match was attributed to physical stress, while in another patient bleeding on the optical disc happened during a migraine attack [22, 23]. Rozenberg studied a large series of 250 eyes with ODD and reported two young patients, aged 4 and 12 years, with submacular hemorrhage.

All types of vascular occlusions have been described in the eyes with ODD and they are generally considered to be caused by vascular compression of the optic nerve head or inside it. They should be treated as the cases without drusen [18, 19]. Submacular hemorrhages in cases with ODD and peripapillary subretinal neovascular membrane generally have a good prognosis for visual acuity and should not be treated by laser. Photocoagulation is indicated only if the visual acuity is compromised [21, 23]. Boldt et al. reported six out of 48 patients with ODD who had vascular occlusion at the level of the optic disc [20, 22–24]. A case of central retinal vein occlusion associated with hormonal contraception was reported.

There is no cure for ODD and the basic approach is monitoring of visual acuity and its loss or following complications such as elevated intraocular pressure or subretinal neovascularization if they develop. Monitoring involves fundus photographs, measuring the thickness of the retinal nerve fiber layer and computerized visual fields. When there are defects in the visual field caused by ODD, regular tonometry and computerized visual fields are mandatory, as these patients are predisposed to have their nerve fibers damaged and they are sensitive to elevated or even normal intraocular pressure [25–28]. Antiglaucomatous drugs should be used in such a case. Ocular antihypertensive agents have been suggested as a prophylaxis to prevent the loss of nerve fibers, and further damage of the optic nerve [27–29].

It is well known that pregnancy causes blood hypercoagulability and pregnant women may be at risk of developing systemic thrombosis and other vascular disorders (Schafer 1985). In patients with large defects in the visual field caused by ODD, a low-dose, systemic, antiplatelet agents can be introduced to prevent possible ischemic events prior to pregnancy [30]. This treatment during pregnancy is safe and can reduce the incidence of bleeding complications [27, 31].

Conclusion

Hemodynamic changes during pregnancy are possible factors affecting the development of optical disc and retinal hemorrhages. Although the literature describes the radial neurotomy fiber optic nerve decompression, there is no established therapeutic treatment for optic disk drusen. Knowing that the treatment of optic disc drusen is limited, the recognition of optic nerve drusen as a cause of hemorrhage during pregnancy prevents unnecessary diagnostic and therapeutic interventions.

References

1. Morris RW, Ellerbrock JM, Hamp AM, Joy JT, Roels P, Davis Jr CN. Advanced visual field loss secondary to optic nerve head drusen: case report and literature review. Optometry 2009;80(2):83-100.
2. Wilkins JM, Pomeranz HD. Visual manifestations of visible and buried optic disc drusen: review. J Neuroophthalmol 2004;24(2):125-9.
3. Optic disc drusen (editorial). Review of optometry. New York: Jobson Publishing, LLC; 2013. p. 75-7.
4. Friedman AH, Beckerman B, Gold DH, Walsh JB, Gartner S. Druse of the optic disc: review. Surv Ophthalmol 1977;21(5):375-90.
5. Auw-Haedrich C, Staubach F, Witschel H. Optic disk drusen: review. Surv Ophthalmol 2002;47(6):515-31.
6. Katz BJ, Pomeranz HD. Visual field defects and retinal nerve fiber layer defects in eyes with buried optic nerve drusen. Am J Ophthalmol 2006;141:248-53.
7. Lee AG, Zimmerman MB. The rate of visual field loss in optic nerve head drusen. Am J Ophthalmol 2005;139:1062-6.
8. Gili P, Flores-Rodríguez P, Yangüela J, Ordu˜na-Azcona J, Martín-Ríos MD. Evaluation of optic disc size in patients with optic nerve head drusen using fundus photography. J Optom 2013;6:75-9.
9. Manzanaro PG, Rodilla JY, Caravaca GR, Font CC, Rodrigo JCR, Puent AA. Decreased visual acuity from optic disc drusen. Arch Soc Esp Oftalmol 2010;85(2):64-9.
10. Ogawa R, Usui T, Takagi M, Hasegawa S, Abe H, Nanba K. Bilateral optic disc drusen with severe progressive visual field defect. Neuroophthalmol 2001;2(4):229-32.
11. Brodrick JD. Drusen of the disc and retinal haemorrhages. Br J Ophthalmol 1973;57:299-306.
12. Lee KM, Woo SJ, Hwang JM. Morphologic characteristics of optic nerve head drusen on spectral-domain optical coherence tomography. Am J Ophthalmol 2013;155:1139-47.
13. Sato T, Mrejen S, Spaide RF. Multimodal imaging of optic disc drusen. Am J Ophthalmol 2013;156:275-82.
14. Floyd MS, Katz BJ, Digre KB. Measurement of the scleral canal using optical coherence tomography in patients with optic nerve drusen. Am J Ophthalmol 2005;139:664-9.
15. Rubinstein K, Ali M. Retinal complications of optic disc drusen. Br J Ophthalmol 1982;66:83-95.
16. Romero J, Sowka J, Shechtman D. Hemorrhagic complications of optic disc drusen and available treatment options. Optometry 2008;79:496-500.
17. Sanders TE, Gay AJ, Newman M. Hemorrhagic complications of drusen of the optic disk. Am J Ophthalmol 1971;71: 204-17.
18. Neffendorf JE, Mulholland C, Quinlan M, Lyons CJ. Disc drusen complicated by optic disc hemorrhage in childhood. Can J Ophthalmol 2010;45:537-8.
19. Abeysiri P, Sivaprasad S, Trew D. Optic disc drusen. Lancet 2003;362(9):468.
20. Silbert Aumiller M. Optic disc drusen: complications and management. Optometry 2007;78:10-6.
21. Nicholson B, Ahmad B, Sears JE. Congenital optic nerve malformations. Int Ophthalmol Clin 2011;51(1):49-76.
22. Zaidi FH, Corbett MC. Optic disc drusen associated with neovascularization of optic disc. Eye 2005;19:814-6.
23. Borruat FX, Sanders MD. Vascular papillary anomalies and complications in optic disc drusen. Klin Monatsbl Augenheilkd 1996;208(5):294-6.
24. Austin JK. Optic disc drusen and associated venous stasis retinopathy. J Am Optom Assoc 1995;66(2):91-5.
25. Flores-Rodriguez P, Gili P, Martin-Rios MD. Sensitivity and specificity of time-domain and spectral-domain optical coherence tomography in differentiating optic nerve head drusen and optic disc oedema. Ophthalmic Physiol Opt 2012;32:213-21.
26. Yehoshua Z, Gregori G, Sadda SR, Penha FM, Goldhardt R, Nittala MG, et al. Comparison of drusen area detected by spectral domain optical coherence tomography and color fundus imaging. Invest Ophthalmol Vis Sci 2013;54(4):2429-34.
27. Mikel M, Chidambaram Y, Amadeo RR. Optic disc drusen and anterior ischaemic optic neuropathy in pregnancy. Neuroophthalmol 2012;36(1):23-5.
28. Rott D, Leibowitz D. Optic nerve head drusen mimicking papilledema and malignant hypertension. Eur J Intern Med 2009;20:112-3.
29. Johnson LN, Diehl ML, Hamm CW, Sommerville DN, Petroski GF. Differentiating optic disc edema from optic nerve head drusen on optical coherence tomography. Arch Ophthalmol 2009;127(1):45-9.
30. Sullu Y, Yıldız L, Erkan D. Submacular surgery for choroidal neovascularization secondary to optic nerve drusen. Am J Ophthalmol 2003;136:367-70.
31. Mehta JS, Bates A, Chan J, Kuteesa W, Acheson JF. Pregnancy associated, optic disc drusen related visual loss. Acta Ophthalmol Scand 2005;271-3.

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– Navesti u koju kategoriju spada rad koji se šalje (originalni naučni rad, pregledni članak, prethodno saopštenje, stručni članak, prikaz slučaja, istorija medicine).

Rukopis

Za pisanje teksta koristiti *Microsoft Word for Windows*. Tekst treba otkucati koristeći font *Times New Roman*, na stranici formata A4, proredom od 1,5 (i u tabelama), sa marginama od 2,5 cm i veličinom slova od 12 pt. Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana. Naslovna strana treba da sadrži kratak i jasan naslov rada, bez skraćenica, zatim kratki naslov (do 40 karaktera), puna imena i prezimena autora (najviše 6 autora) indeksirana brojkama koje odgovaraju onima kojim se u zaglavlju navode uz pun naziv i mesta ustanova u kojima autori rade. Na dnu ove stranice navesti titulu, punu adresu, e-mail i broj telefona ili faksa autora zaduženog za korespondenciju.

2. Sažetak. Sažetak treba da sadrži do 250 reči, bez skraćenica, sa preciznim prikazom problematike, ciljeva, metodologije, glavnih rezultata i zaključaka. Sažetak treba da ima sledeću strukturu:

– originalni naučni radovi: uvod (sa ciljem rada), materijal i metode, rezultati i zaključak;

– prikaz slučaja: uvod, prikaz slučaja i zaključak;

– pregled rada: uvod, odgovarajući podnaslovi koji odgovaraju onima u tekstu rada i zaključak.

U nastavku navesti do deset ključnih reči iz spiska medicinskih predmetnih naziva (*Medical Subjects Headings, MeSH*) Američke nacionalne medicinske biblioteke.

3. Sažetak na engleskom jeziku. Sažetak na engleskom jeziku treba da bude prevod sažetka na srpskom jeziku, da ima istu strukturu i da sadrži do 250 reči, bez upotrebe skraćenica.

4. Tekst rada

– Tekst originalnih članaka mora da sadrži sledeće celine:

Uvod (sa jasno definisanim ciljem rada), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su korišćene u tekstu) i eventualna zahvalnost autora onima koji su pomogli u istraživanju i izradi rada.

– Tekst prikaza slučaja treba da sadrži sledeće celine: Uvod (sa jasno definisanim ciljem rada), Prikaz slučaja, Diskusija i Zaključak.

– Tekst treba da bude napisan u duhu srpskog jezika, oslobođen suvišnih skraćenica, čija prva upotreba zahteva navođenje punog naziva. Skraćenice ne upotrebljavati u naslovu, sažetku i zaključku. Koristiti samo opšte prihvaćene skraćenice (npr. DNA, MRI, NMR, HIV,...). Spisak skraćenice koje se navode u radu, zajedno sa objašnjenjem njihovog značenja, dostaviti na poslednjoj stranici rukopisa.

– Koristiti mere metričkog sistema prema Internacionalnom sistemu mera (*International System Units – SI*). Temperaturu izražavati u Celzijusovim stepenima (°C), a pritisak u milimetrima živinog stuba (mmHg).

– Ne navoditi imena bolesnika, inicijale ili brojeve istorija bolesti.

Uvod sadrži precizno definisan problem kojim se bavi studija (njegova priroda i značaj), uz navođenje relevantne literature i sa jasno definisanim ciljem istraživanja i hipotezom.

Materijal i metode treba da sadrže podatke o načinu dizajniranja studije (prospektivna/retrospektivna, kriterijumi za uključivanje i isključivanje, trajanje, demografski podaci, dužina praćenja). Statističke metode koje se koriste treba da budu jasne i detaljno opisane.

Rezultati predstavljaju detaljan prikaz podataka dobijenih tokom studije. Sve tabele, grafikoni, sheme i slike moraju da budu citirani u tekstu, a njihova

numeracija treba da odgovara redosledu pominjanja u tekstu.

Diskusija treba da bude koncizna i jasna, sa interpretacijom osnovnih nalaza studije u poređenju sa rezultatima relevantnih studija publikovanim u svetskoj i *domaćoj* literaturi. Navesti da li je hipoteza istraživanja potvrđena ili opovrgnuta. Izneti prednosti i ograničenja studije.

Zaključak u kratkim crtama mora da odbaci ili potvrdi pogled na problem koji je naveden u Uvodu. Zaključci treba da proizilaze samo iz vlastitih rezultata i da ih čvrsto podržavaju. Uzdržati se uopštenih i nepotrebnih zaključivanja. Zaključci u tekstu moraju suštinski odgovarati onima u Sažetku.

5. Literatura. Literatura se u tekstu označava arapskim brojevima u uglastim zagradama, prema redosledu pojavljivanja. Izbegavati veliki broj citata u tekstu. Za naslove koristiti skraćenice prema *Index Medicus*-u (http:// www.nlm.nih.gov/tsd/serials/lji.html). U popisu citirane literature koristiti Vankuverska pravila koja precizno određuju redosled podataka i znake interpunkcije kojima se oni odvajaju, kako je u nastavku dato pojedinim primerima. Navode se svi autori, a ukoliko ih je preko šest, navesti prvih šest i dodati et al.

Članci u časopisima:

∗ *Standardni članak*

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

∗ *Organizacija kao autor*

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

∗ *Nisu navedena imena autora*

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

∗ *Volumen sa suplementom*

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

∗ *Sveska sa suplementom*

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

∗ *Sažetak u Časopisu*

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

Knjige i druge monografije:

∗ *Jedan ili više autora*

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

∗ *Urednik(ci) kao autor*

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

∗ *Poglavlje u knjizi*

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

∗ *Rad u zborniku radova*

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.

∗ *Disertacije i teze*

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

Elektronski materijal

∗ *Članak u Časopisu u elektronskoj formi*

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

∗ *Monografije u elektronskoj formi*

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

∗ *Kompjuterski dokument (file)*

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

6. Prilozi (tabele, grafikoni, sheme i fotografije).

Dozvoljeno je najviše šest priloga!

– Tabele, grafikoni, sheme i fotografije dostavljaju se na kraju teksta rukopisa, kao posebni dokumenti na posebnim stranicama.

– Tabele i grafikone pripremiti u formatu koji je kompatibilan sa programom *Microsoft Word for Windows.*

– Slike pripremiti u JPG, GIF TIFF, EPS i sl. formatu

– Svaki prilog numerisati arapskim brojevima, prema redosledu njihovog pojavljivanja u tekstu.

– Naslov, tekst u tabelama, grafikonima, shemama i legendama navesti na srpskom i na engleskom jeziku.

– Objasniti sve nestandardne skraćenice u fusnotama koristeći sledeće simbole: *, †, ‡, §, ||, ¶, **, ††, ‡‡.

– U legendama mikrofotografija navesti korišćenu vrstu bojenja i uvećanje na mikroskopu. Mikrofotografije treba da sadrže merne skale.

– Ukoliko se koriste tabele, grafikoni, sheme ili fotografije koji su ranije već objavljeni, u naslovu navesti izvor i poslati potpisanu izjavu autora o saglasnosti za objavljivanje.

– Svi prilozi biće štampani u crno-beloj tehnici. Ukoliko autori žele štampanje u boji potrebno je da snose troškove štampe.

7. Slanje rukopisa

Prijem rukopisa vrši se u elektronskoj formi na stranici: **aseestant.ceon.rs/index.php/medpreg/**. Da biste prijavili rad morate se prethodno registrovati. Ako ste već registrovani korisnik, možete odmah da se prijavite i započnete proces prijave priloga u pet koraka.

8. Dodatne obaveze

Ukoliko autor i svi koautori nisu uplatili članarinu za Medicinski pregled, rad neće biti štampan. Radovi koji nisu napisani u skladu sa pravilima Medicinskog pregleda, neće biti razmatrani. Recenzija će biti obavljena najkasnije u roku od 6 nedelja od prijema rada. Uredništvo zadržava pravo da i pored pozitivne recenzije donese odluku o štampanju rada u skladu sa politikom Medicinskog pregleda. Za sva dodatna obaveštenja obratiti se tehničkom sekretaru:

Društvo lekara Vojvodine
Vase Stajića 9
21000 Novi Sad
Tel. 021/521 096; 063/81 33 875
E-mail: dlv@neobee.net

INFORMATION FOR AUTHORS

Medical review publishes papers from various fields of biomedicine intended for broad circles of doctors. The papers are published in Serbian language with an expanded summary in English language and contributions both in Serbian and English language, and selected papers are published in English language at full length with the summary in Serbian language. Papers coming from non-Serbian speaking regions are published in English language. The authors of the papers have to be Medical Review subscribers.

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

The covering letter:

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

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

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

The manuscript:

Use Microsoft Word for Windows to type the text. The text must be typed in font Times New Roman, page format A4, space 1.5 (for tables as well), borders of 2.5 cm and font size 12pt. The manuscript should contain the following elements:

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

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

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

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

– review papers should have the introduction, subtitles corresponding to those in the paper and conclusion. It is to be followed by up to 10 Key Words from the list of Medical Subject Headings, MeSH of the American National Medical Library.

3. The summary in Serbian language. The summary in Serbian should be the translation of the summary in English, it should be structured in the same way as the English summary, containing up to 250 words, without any abbreviations.

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

– The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

– The text should be written in the spirit of Serbian language, without unnecessary abbreviations, whose first mentioning must be explained by the full term they stand for. Abbreviations should not be used in the title, summary and conclusion. Only commonly accepted abbreviations (such as DNA, MRI, NMR, HIV…) should be used. The list of abbreviations used in the text, together with the explanation of their meaning, is to be submitted at the last page of the manuscript.

– All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C). and pressure in mmHg.

– No names, initials or case history numbers should be given.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Material and methods should contain data on design of the study (prospective/retrospective, eligibility

and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the problem mentioned in the introduction. Conclusions must be based solely on the author's own results, corroborating them. Avoid generalised and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

5. References. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by et 'al'.

Articles in journals

∗ *A standard article*

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

∗ *An organisation as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *Acomputer file*

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

6. Attachments (tables, graphs, schemes and photographs). The maximum number of attachments allowed is six!

– Tables, graphs, schemes and photographs are to be submitted at the end of the manuscript, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English language.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, ||, ¶, **, † †, ‡ ‡ .

– State the type of colour used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in colour, they will have to pay additional cost.

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The manuscripts can be submited on the web-page: **aseestant.ceon.rs/index.php/medpreg/**. The authors have to register with the journal prior to submitting their manuscript, or, if already registered, they can simply log in and begin the 5 step process.

8. Additional requirements

If the author and all co-authors have failed to pay the subscription for Medical Review, their paper will not be published.

Papers which have not met the criteria of Medical Review will not be taken into consideration. The Editorial review of the paper will be announced not later than six weeks after the submission of the paper. The Editorial Board reserves the right to make a decision regarding the publication of the paper according to the policy of Medical Review even if the review is positive. Contact the technical secretary for all additional information:

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E-mail: dlv@neobee.net