Publishing Sector of the Society of Physicians of Vojvodina of the Medical Society of Serbia, Novi Sad, Vase Stajica 9,

MEDICAL REVIEW

JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
THE FIRST ISSUE WAS PUBLISHED IN 1948

MEDICAL REVIEW is published two-monthly (six double issues per a year) in the circulation of 1000 copies. Payment for individuals from the territory of Serbia for the year 2016 is 3,000.00 dinars (the VAT being calculated in) and 4,000.00 dinars for the individuals outside the territory of Serbia, and 8,000.00 dinars (+ the VAT) for institutions. The payments are to be made to the account number 340-1861-70 or 115-13858-06, with the remark "Additional membership fee for the Medical Review".

The manuscripts can be submited on the web-page: aseestant.ceon.rs/index.php/medpreg/.
Address Editorial: Društvo lekara Vojvodine Srpskog lekarskog društva, 21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875, E-mail: dlv@neobee.net; Web: www.dlv.org.rs

Printed by: "Feljton" Novi Sad

Izdavačka delatnost Društva lekara Vojvodine Srpskog lekarskog društva, Novi Sad, Vase Stajića 9

MEDICINSKI PREGLED

ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
PRVI BROJ JE ŠTAMPAN 1948. GODINE.

MEDICINSKI PREGLED izlazi dvomesečno (šest dvobroja godišnje), u tiražu od 1000 primeraka. Pretplata za pojedince sa teritorije Srbije za 2016. godinu iznosi 3.000,00 dinara (sa uračunatim PDV-om), a 4.000,00 dinara za pojedince van teritorije Srbije, a za ustanove 8.000,00 dinara (uz dodavanje PDV-a). Uplate se vrše na račun broj 340-1861-70 ili 115-13858-06, s naznakom „Dodatna članarina za Medicinski pregled".

Prijem rukopisa vrši se u elektronskoj formi na stranici: aseestant.ceon.rs/index.php/medpreg/.
Adresa Redakcije: Društvo lekara Vojvodine Srpskog lekarskog društva,
21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875
E-mail: dlv@neobee.net; Web: www.dlv.org.rs

Štamparija: »Feljton« Novi Sad

M E D I C A L R E V I E W
JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
Novi Sad Vase Stajića 9 Serbia

Med Pregl 2016; LXIX (7-8): 193-262. Novi Sad: July-August.

CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad Vase Stajića 9 Srbija

Med Pregl 2016; LXIX (7-8): 193-262. Novi Sad: juli-avgust.

SADRŽAJ

ORIGINALNI NAUČNI RADOVI

PRIKAZI SLUČAJEVA

SEMINAR ZA LEKARE U PRAKSI

ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

Clinical Center of Vojvodina, Center for Laboratory Medicine, Novi Sad[1]
University of Novi Sad, Faculty of Medicine, Department of Pathophysiology[2]
Institute for Public Health of Vojvodina, Novi Sad, Serbia[3]

Original study
Originalni naučni rad
UDK 616.63-074/-076
DOI: 10.2298/MPNS1608197C

THE LABORATORY ASPECTS OF PROTEINURIA

LABORATORIJSKI ASPEKTI PROTEINURIJE

Velibor ČABARKAPA[1,2], Mirjana ĐERIĆ[1,2], Branislava ILINČIĆ[1,2], Biljana VUČKOVIĆ[1,2], Aleksandra TRIFU[1] and Mirko ŠIPOVAC[3]

Summary

Introduction. The existence of proteinuria may be overlooked by applying the test strips. The aim of this study has been to determine the discrepancy between the findings of proteinuria detected by test strips when compared to the results of its testing with the sulfosalicylic acid. **Material and Methods.** The study sample consisted of 1106 subjects, who were divided into the proteinuria positive (test strips showed the presence of isolated proteinuria), and proteinuria negative group (microscopic examination revealed the presence of ≥10 fresh red blood cells/μL, and/or ≥1 dysmorphic erythrocyte/μL, and/or ≥10 leukocytes/μL, and/or≥ 1 cylinder, and/or ≥1 nonsquamous epithelial cells/μL, and/or ≥100 bacteria/μL). Both groups had the urine tested with sulfosalicylic acid. The chemical and microscopic examination of the urine was done by the analyzer LabUMat-UriSed. **Results.** Proteinuria was confirmed with the sulfosalicylic acid test in 96.5% of subjects from group 1 and in 85.3% of subjects from group 2. Among the patients with the negative finding of proteinuria on the test strip and with the positive sulfosalicylic acid test there was a significantly higher number of those with pathological findings of erythrocytes, leukocytes, bacteria and cylinders in the urine when compared to those of the same group with negative sulfosalicylic acid test. **Conclusion.** Sulfosalicylic acid test should be performed in cases of pathological microscopic findings in the urine in case of the presence of ≥10 fresh erythrocytes/μL and/or ≥1 dysmorphic erythrocyte/μL and/or ≥10 leukocytes/μL and/or ≥1 cylinder (except hyaline) and/or ≥1 nonsquamous epithelial cells/μL and/or ≥100 bacteria/μL even if the test strip examination is negative for proteinuria.

Key words: Proteinuria; Reagent Strips; Urinalysis; Salicylates; Morphological and Microscopic Findings; Urine Specimen Collection

Sažetak

Uvod. Postojanje proteinurije se može prevideti upotrebom test-trake. Cilj ove studije je da se utvrdi diskrepanca između nalaza proteinurije otkrivene test-trakama u odnosu na rezultate testiranja sa sulfosalicilnom kiselinom. **Materijal i metode.** Ukupno 1 106 ispitanika, podeljeno je u grupu pozitivnih na proteinuriju (prisustvo izolovane proteinurije na test-traci), kao i na grupu negativnih na proteinuriju (mikroskopski pregled otkriva prisustvo ≥ 10 svežih eritrocita/μl , i/ili ≥ 1 dismorfični eritrocit /μl, i/ili ≥ 10 leukocita /μl, i / ili ≥ 1 cilindar, i/ili ≥ 1 neskvamozna epitelna ćelija/μl, i/ili ≥ 100 bakterija /μl). U obe grupe, urin je testiran sulfosalicilnom kiselinom. Hemijski i mikroskopski pregled urina vršen je na analizatoru LabUMat. **Rezultati.** Testom sulfosalicilnom kiselinom proteinurija je potvrđena kod 96,5% ispitanika prve grupe, kao i kod 85,3% ispitanika druge grupe. Kod ispitanika sa negativnim nalazom proteinurije na test-traci i sa pozitivnim testom sa sulfosalicilnom kiselinom postoji znatno veći broj onih sa patološkim nalazima eritrocita, leukocita, bakterija i cilindara u urinu u odnosu na ispitanike iste grupe sa negativnim testom sa sulfosalicilnom kiselinom. **Zaključak.** Testiranje sulfosalicilnom kiselinom trebalo bi primenjivati u slučaju patološkog mikroskopskog nalaza u urinu ukoliko postoji prisustvo ≥ 10 svežih eritrocita/μl i/ili ≥ 1 dismorfičnih eritrocita/μl i/ili ≥ 10 leukocita/μl i/ili ≥ 1 cilindra (osim hijalinih) i/ili ≥ 1 neskvamoznih epitelnih ćelija/μl i/ili ≥ 100 bakterija/μl, čak i ako je nalaz proteinurije na test-trakama negativan.

Ključne reči: proteinurija; test trake; analiza urina; salicilati; morfološki i mikroskopski nalazi; sakupljanje uzorka urina

Introduction

Kidney diseases often develop asymptomatically and may remain undetected until advanced stages of renal insufficiency have developed, or they may be discovered incidentally in the early stages of the disease, based on the examination of the first

Acknowledgments

This work was supported by grant No 114-451-1809/2014 approved by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, Republic of Serbia

Corresponding Author: Doc. dr Velibor Čabarkapa, Klinički centar Vojvodine, Centar za laboratorijsku medicinu, 210000 Novi Sad, Hajduk Veljkova 1-7, E-mail: veliborcabarkapa@gmail.com

Abbreviations

A/C – albumin/creatinine ratio
AUA – asymptomatic urinary abnormalities
CKD – chronic kidney disease
CCV – Clinical Center of Vojvodina
GFR – glomerular filtration rate
ESRD – end stage renal disease
NEC – nonsquamos epithelial cells
P/C – protein/creatinine ratio
SDS – Na-dodecyl sulfate
SSA – sulfosalicylic acid

morning urine, usually as a part of a systematic examination or during health check-ups for employees [1]. The presence of hematuria and/or proteinuria in the absence of any obvious impairment of the kidney function as well as any symptom or sign of kidney disease is referred to as asymptomatic urinary abnormalities (AUA) [2]. To confirm AUA it is necessary to repeat the examination of the first morning urine and to perform additional diagnostic procedures in order to detect kidney disease. It is of great importance to identify this category of patients so as to provide them the prompt therapy and thus delay the progression of the disease.

However, praxis shows that both doctors and patients rarely pay full attention to AUA. This is partly due to certain errors that occur before performing the laboratory tests (pre analytical phase) such as improper collection of the urine and untimely delivering of the urine samples to the laboratory, and errors that are related to the phase of the analysis (the analytical phase) due to the presence of various interfering factors, some analytical limitations, etc. A good part of these errors can be contributed to the inadequate cooperation between patients and doctors.

Proteinuria can be a harmless finding if it is detected in only one urine sample, while persistent proteinuria may indicate the presence of a kidney disease. Proteinuria associated with erythrocyturia indicates glomerulopathy while proteinuria associated with leukocyturia, bacteriuria and non-squamous epithelial cells (NEC) indicates a tubulointerstitial disease. In addition, albuminuria as a marker of kidney impairment is a parameter for diagnosing the first stage of chronic kidney disease (CKD), even in terms of a preserved glomerular filtration rate (GFR) [3]. Besides, persistent albuminuria is a characteristic finding for initial diabetic nephropathy.

The standard method to detect proteinuria is chemical testing of the first morning urine using test strips. However, most test strips, even of different manufacturers, are mainly based on the principle of detecting albumin, and therefore the glomerular type of proteinuria. In addition, test strips can give false positive results such as in alkaline urine, highly concentrated urine, hematuria, when using certain drugs, etc., or false negative results such as in diluted urine (relative density <1.015) or when the urinary proteins are of non-albumin nature [4]. Indirect evidence of proteinuria is the presence of cylinders in the microscopic examination of urine, while the chemical test with sulfosalicylic acid (SSA) is a confirming test for the presence of proteinuria.

Since it is assumed that the existence of proteinuria may be overlooked by applying test strips, the aim of this study has been to determine the discrepancy between the findings of proteinuria detected by chemical examination of urine by test strips when compared to the results of its testing with the SSA. Moreover, in a number of samples with the mentioned discrepancy, quantitative tests were used to determine the degree of proteinuria and to determine the predominant type of proteinuria.

Material and Methods

This study was conducted at the Clinical Center of Vojvodina (CCV), Novi Sad by reviewing the medical records of the routine examination of the first morning urine in the period from October 2014 to February 2015. Out of 6835 examined patients (aged over 18 years), 1106 subjects were included in the study based on the finding of proteinuria which was detected by the chemical examination of urine using test strips and by the finding of the microscopic examination of the urine. The subjects were divided into two groups. Group 1 (proteinuria positive respondents) consisted of patients in whom the test strips showed the presence of isolated proteinuria (proteinuria positive results on the test strips with a normal chemical and microscopic examination of urine). Group 2 (proteinuria negative subjects) consisted of subjects whose chemical examination done by means of test strips was negative for the presence of proteins but the microscopic examination revealed the presence of ≥10 fresh red blood cells/μL (reference interval 0-5/μL) and/or ≥1 dysmorphic erythrocyte/μL (reference interval 0-0/μL) and/or ≥10 leukocytes/μL (reference interval 0-5/μL) and/or ≥1 cylinder (except hyaline) (reference range 0-0/μL, and for the hyaline cylinders the reference interval 0-1/μL)) and/or ≥1 NEC/μL(reference interval 0-3/μL), and/or ≥100 bacteria/μL (reference interval for the bacteria is 0-20 /μL).

The urine of patients from both groups was tested with SSA; in group 1 it was used as a confirming test for the presence of isolated proteinuria, and in the other group as a complementary diagnostic test for detecting proteinuria. The test was performed by adding 5-10 drops of 20% SSA in about 5 ml of urine. Depending on the quantity of proteins present in the urine, cloudiness i.e. turbidity of some degree occurs. SSA test result was expressed either as "negative reaction with SSA" (corresponding to <0.1 g/L) or if it was positive: barely noticeable turbidity (corresponding to about 0.2 g/L), 1+ (corresponding to 0.3-1 g/L), 2+ (corresponding to 1-2.5 g/L), 3+ (corresponding to 2.5-4.5 g/L), or 4+ (corresponding to >4.5 g/L). Result reading of the SSA test was performed by a single laboratory technician with years of experience.

Table 1. The results of the chemical examination of urine using test strips for detecting proteinuria and the sulfosalicylic acid test (SSA) in all subjects
Tabela 1*. Rezultati hemijskog pregleda urina test trakom na prisustvo proteinurije i testa sa sulfosalicilnom kiselinom (SSA) kod svih ispitanika*

Proteinuria – chemical examination of urine via test strips *Hemijski pregled urina test trakom*	N	SSA+ n (%)	SSA- n (%)	p
Negative/*Negativno*	962	821 (85.3%)	141 (14.7%)	<0.001
Positive/*Pozitivno*	144	139 (96.5%)	5 (3.5%)	<0.001

Legend: SSA+: positive sulfosalicylic acid test; SSA- : negative sulfosalicylic acid test; N (n) - the number of subjects
Legenda: *SSA+: pozitivan test na sulfosalicilnu kiselinu; SSA-: negativan test na sulfosalicilnu kiselinu; N (n)- broj ispitanika*

All subjects underwent chemical and microscopic examination of the first morning urine by the automated analyzer LabUMat-UriSed (77 ElektronikaKft, Hungary) at the Center for Laboratory Medicine of CCV. The chemical examination of urine was done with LabStrip U11 Plus test strips (77 ElektronikaKft., Hungary) as each strip has 11 test fields with reagents for the determination of 11 standard parameters for the chemical examination of urine. The microscopic examination of the urine was performed by the flow cytometry method after the chemical examination, without prior centrifugation of the sample.

In 29 patients in whom the chemical examination using test strips was negative for proteinuria but the SSA test was positive, quantitative tests were performed to measure the concentration of proteins, albumin and creatinine, and to determine the type of proteinuria in the same sample of the first morning urine. The following ratios were also calculated: the protein/creatinine (P/C) ratio (mg/mmol), and the albumin/creatinine ratio (A/C) (mg/mmol). The P/C ratio above 22 mg/mmol and the A/C ratio above 3.0 mg/mmol were considered to be a pathological finding [3, 5]. The concentration of protein in the urine was determined by the turbidimetric method with benzethonium chloride, and the level of creatinine was determined by the Jaffe method with alkaline picrate, all by means of commercial kits (Abbott, Wiesbaden, Germany) on the biochemical analyzer ci4000 Architect. The concentration of albumin in the urine was determined by the immunoturbidimetric biochemical analyzer ADVIA 1800 with commercial kits (Siemens Healthcare Diagnostics, Germany). The type of proteinuria was determined using commercial agarose gels HydraGel 5 Proteinurie (Sebia, France). After adding the anionic detergent (Na-dodecyl sulfate (SDS)) proteins form complexes with SDS hence disrupting the native conformation of the proteins and a new one is established with the same conformation and the same negative charge per mass unit. Proteins were then separated by electrophoresis according to their molecular weights.

Statistical analysis was performed by means of STATISTICA 12.0 statistical software (StatSoft, Inc., Tulsa, OK, USA), for which the University of Novi Sad has the university license. The results were presented in tables and graphs. Chi-square test showed the differences between individual variables. A p value of <0.05 was considered statistically significant.

Results

The study included 1106 patients, 507 men and 599 women, their age being 55.3 ± 17.1 years.

Isolated proteinuria (positive finding on the test strip with a normal remaining chemical and microscopic examination of urine) was detected in 144 subject (13%) and proteinuria on the test strip was negative in the remaining 962 persons (87%) despite the pathological microscopic findings (the presence of ≥10 fresh erythrocytes/μL and/or ≥1 dysmorphic erythrocyte/μL and/or≥10 leukocytes/μL and/or ≥1 cylinder (except hyaline) and/or ≥1 NEC/μL and/or ≥100 bacteria/μL.

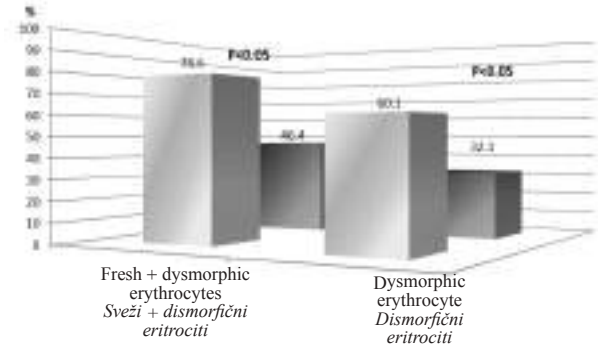
Proteinuria was confirmed with the SSA test in 96.5% of the proteinuria positive subjects from group 1 **(Table 1)**. The SSA test was positive in 85.3% of proteinuria negative subjects, which is significantly higher in relation to the negative SSA testing (p <0.001).

Among the patients with the negative finding of proteinuria on the test strip and with the positive SSA test there is a significantly higher number of those with pathological findings of erythrocytes in the urine when compared to those of the same group with negative SSA test, in the form of an increased number of fresh erythrocytes and/or the presence of dysmorphic erythrocytes, as well as the isolated presence of dysmorphic erythrocytes (p <0.05) **(Graph 1)**.

Among the subjects with negative findings of proteinuria on the test strip examination but with positive findings for it when tested with SSA there was a significantly higher number of those with pathological findings of leukocytes (> 10/μL), bacteria (> 100 /μL) and cylinders (> 1/μL) in the urine when compared to the same group of subjects with the negative test with SSA (p <0.05), while the number of patients with NEC> 1/μL was significantly higher among those with the negative SSA test **(Graph 2)**.

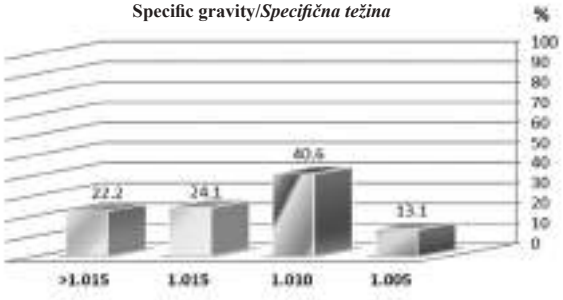
The specific gravity of the urine ≥1.015 was present as a potential cause of false-negative results on the test strip in almost half of all subjects (46.1%) with negative findings of proteinuria on the test strip examination and with positive findings on the SSA test **(Graph 3).**

In the subgroup of 29 patients with negative proteinuria on the test strip examination and positive SSA test, it was observed that an abnormal A/C ratio accompanied with pathological P/C ratio was present



Graph 1. The frequency (%) of the pathological number of erythrocytes in the urine
Grafikon 1. *Učestalost (%) patološkog broja eritrocita u urinu*
Legend: SSA+: positive sulfosalicylic acid test; SSA-: negative sulfosalicylic acid test
Legenda: *SSA+: pozitivan test na sulfosalicilnu kiselinu; SSA: negativan test na sulfosalicilnu kiselinu*

in 15 subjects (51.7%), abnormal A/C ratio with the normal P/C ratio was present in 2 subjects (6.9%), while A/C and the P/C ratio was within the limits of the reference range in 12 subjects. Tubular type of proteinuria was present in 14 subjects; mixed type in only 1 subject, the findings pointed to the presence

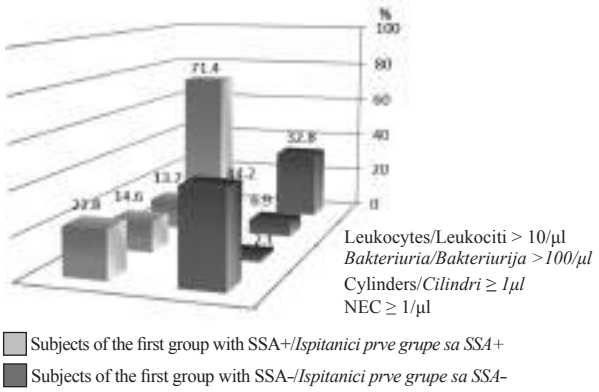


Graph 3. The specific gravity of urine in patients with negative findings of proteinuria on the test strip examination and a positive sulfosalicylic acid test
Grafikon 3*. Specifična težina urina kod ispitanika sa negativnim nalazom proteinurije na test traci i pozitivnim testom sa sulfosalicilnom kiselinom*

of microalbuminuria in 2 subjects and they were negative for proteinuria on the agarose gels test in 12 subjects **(Figure 1)**.

Discussion

Proteinuria represents protein excretion in the urine in a quantity exceeding 150 mg/day [6]. This occurrence is benign, i.e. it is a harmless finding



Graph 2. The frequency (%) of pathological leukocyte count, bacteria, cylinders and nonsquamous epithelial cells in the urine of patients with negative findings of proteinuria on the test strip examination (the second group of respondents).
Grafikon 2. *Učestalost (%) patološkog broja leukocita, bakterija, cilindara i neskvamoznih epitelnih ćelija (NEC) u urinu kod ispitanika sa negativnim nalazom proteinurije na test traci (druga grupa ispitanika)*
Legend: SSA+: positive sulfosalicylic acid test; SSA-: negative sulfosalicylic acid test
Legenda*: SSA+: pozitivan test na sulfosalicilnu kiselinu; SSA: negativan test na sulfosalicilnu kiselinu*

when it is caused by dehydration, fever, severe emotional stress, inflammatory processes of the genitourinary tract (of a non-kidney origin), intensive physical activity, postural mechanisms, or due to exposure to low temperature [7]. Therefore, if the results of the examination of the first morning urine are abnormal, it must be a routine to examine new urine samples after the cessation of conditions that may lead to benign proteinuria.

However, persistent proteinuria indicates the presence of a renal disease, such as glomerular diseases

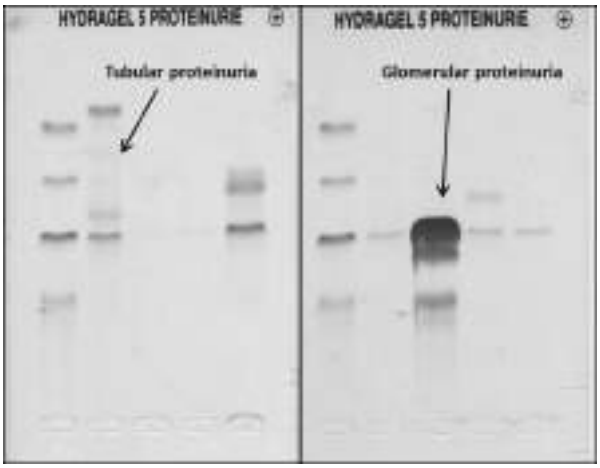


Figure 1. Examples of tubular and glomerular types of proteinuria on the HydraGel 5 proteinuria gels
Slika 1. *Primeri tubulskog i glomerulskog tipa proteinurije na Hydragel 5 proteinurije gelovima*

as a part of the primary and secondary glomerulopathies, and tubular diseases as a part of the tubulointerstitial nephropathies such as those caused by the use of some drugs (non-steroidal anti-inflammatory drugs, antibiotics, etc.), pyelonephritis, hypokalemia, hypercalcemia and others. In addition, proteinuria is one of the most important predictors of CKD progression [8, 9]. Increased and persistent protein reabsorption in tubulocytes leads to the damage of these cells and impairs their function [10].

The first step in the detection of proteinuria is the chemical analysis of the urine using test strips, which is based on the "protein error" of the pH indicators, or the capability of proteins to change the color of some acid-base indicators without changing the pH [4]. The sensitivity of the test strip for protein depends on the manufacturer and it ranges from 0.1-0.3 g/L [4]. The test strips are usually sensitive to the glomerular type of proteinuria because they are sensitive to the presence of albumin [11], while they are insensitive to proteins of non-albumin nature, which could be found in urine such as the light chains of immunoglobulins. There are numerous interfering factors that can lead to false positive and false negative results.

Test strips may give false positive results as it can be in case of alkaline urine (pH > 7.5), if the test strip is held submerged in urine for too long, in highly concentrated urine, intense hematuria, when using penicillin, sulfonamides and other drugs, in the presence of pus, seed or vaginal secretions. False-negative results occur in dilute urine (specific gravity <1.015) or when the urinary proteins are of non-albumin nature, i.e. of low molecular weight [4, 12].

If the test strip indicates the presence of protein in the urine, it is recommended to do one of the confirmatory tests [4, 13], which implies that the confirmatory test has to have at least the same sensitivity and better specificity than the primary test. In laboratory practice, the preference is to use the SSA test, which is based on the chemical precipitation of all proteins [14]. The sensitivity of the SSA test is 0.1 g/L. However, with the use of the SSA it is possible to obtain false-positive results (use of contrast agents in radiology, high concentrations of antibiotics - penicillin, cephalosporins, present turbidity in the urine, which is why the SSA test is performed in centrifuged urine) and false negative results (highly buffered alkaline urine which neutralizes the SSA reagent, diluted urine, turbid urine) [15].

In this study, proteinuria was confirmed with the SSA test in 96.5% of proteinuria positive subjects from group 1, and in only 5 (3.5%) the SSA test was negative, which may be due to false negative SSA test results, or false positive test strip results.

However, proteinuria may be present despite the negative test strip results [16]. Proteinuria is a frequent companion of hematuria in glomerular kidney diseases, leukocyturia and bacteriuria in inflammatory diseases of the kidney and some tubulopathies. In addition, the presence of proteins in the urine is the starting point for creating cylinders [6]. Therefore, we have established criteria to perform the SSA test when the test strip examination was negative in those subjects who had pathological findings in the microscopic examination of the urine. The criteria applied in this study relate to the presence of ≥10 fresh erythrocytes/μL and/or ≥1 dysmorphic erythrocyte/μL and/or ≥10 leukocytes/μL and/or ≥1 cylinder (except hyaline) and/or ≥ 1 NEC/μL and/or ≥100 bacteria/μL. We have found that the SSA test was positive in 85.3% of the subjects, which is significantly higher than the number of subjects with a negative SSA test ($p < 0.001$). Besides, a significant number of these subjects had erythrocyturia (>70%) and leukocyturia (>70%), which was also significantly higher than in the group of the subjects with negative SSA test ($p < 0.001$). In addition, cylindruria was present in a significant number of these subjects when compared to the number of those with negative SSA test ($p < 0.05$), as opposed to the pathological findings NEC which were more frequent in subjects with negative SSA test ($p < 0.05$). False negative results of proteinuria obtained by chemical testing using test strips may be due to diluted urine (relative density < 1.015). In these subjects (with false negative proteinuria) 56% of the samples had a relative density less than 1.015 which is most likely the most common cause of the discrepancy between the chemical examination of urine by test strips when compared to the use of the SSA test.

Another cause of false negative results of proteinuria when using test strips is the presence of proteins of non-albumin nature, i.e. low molecular weight proteins. After confirming the presence of pathological proteinuria via a confirmative test such as the SSA test, it is necessary to determine the P/C and/or A/C ratio in the first morning urine sample or the excretion of protein/albumin in the 24 hour collected urine sample and possibly determine the type of proteinuria with electrophoretic techniques [17, 18]. In a group of 29 such subjects (with test strip negative and SSA positive proteinuria), 15 subjects had abnormal P/C ratio, while in most, tubular type of proteinuria was confirmed using electrophoretic techniques.

Conclusion

Examination of the first morning urine should be carried out by automated analyzers because centrifuging of the sample is avoided after being chemically examined by means of test strips, thus avoiding the loss of certain elements in the urine. Elements that indicate kidney damage in the microscopic examination of urine are kidney tubular cells, erythrocytes, leukocytes, granulated, wide cylinders and dysmorphic erythrocytes [19].

However, it should be kept in mind that potential errors may occur mainly due to poor cooperation between clinicians and patients. Clinicians should give clear instructions on how to collect a urine sample correctly, verify the patient's compliance, and must properly take the anamnesis related to all potentially interfering factors. Another thing that

should be kept in mind is that the sensitivity of the test strips is not very high, that it depends on the manufacturer, and that it is mainly based on the determination of albumin. Therefore, we believe that the confirmatory sulfosalicylic acid test should be performed in all cases of pathological microscopic findings in the urine (presence of ≥10 fresh erythrocytes/μL and/or ≥1 dysmorphic erythrocyte/μL and/or ≥10 leukocytes/μL and/or ≥1 cylinder (except hyaline) and/or ≥1 nonsquamos epithelial cells/μL and/or ≥100 bacteria/μL) even if the test strip examination is negative for proteinuria.

References

1. Đukanović Lj. Ispitivanje bolesnika sa oboljenjima bubrega. U: Đukanović Lj, Oštrić, urednici. Bolesti bubrega. Beograd: Zavod za izdavanje udžbenika i nastavna sredstva; 1999.

2. Bakr A, Sarhan A, Hammad A, Ragab M, Salama O, Al-Husseini F, et al. Asymptomatic urinary abnormalities among primary school children in Egypt. World J Pediatr 2007;3:214-7.

3. KDIGO 2012 Clinical Practice Guideline for the Evaluation and management of Chronic Kidney Disease. Kidney Int Suppl. 2013;1:1-150.

4. Lalić N, Ilić M. Klinički značaj analize urina: atlas sedimenta urina. Beograd: Društvo medicinskih biohemičara srbije i Crne Gore; 2005.

5. Nacionalni vodič dobre kliničke prakse za prevenciju, dijagnostikovanje i lečenje hronične bolesti bubrega. Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse. Ministarstvo zdravlja Republike Srbije. Beograd: Agencija za akreditaciju zdravstvenih ustanova; 2013.

6. Stošić Z. Patofiziologija bubrega. U: Osnovi kliničke patofiziologije. Stošić Z, Borota R, urednici. Novi Sad: Medicinski fakultet; 2013.

7. Carroll M, Temte J. Proteinuria in adults: a diagnostic approach. Am Fam Physician. 2000;62:1333-40.

8. Praga M. Slowing progression of renal failure. Kidney Int. 2002;61(Suppl 2):63-6.

9. Rossert JA, Wauters JP. Recommendations for the screening and management of patients with chronic kidney disease. Nephrol Dial Transplant. 2002;17(Suppl 1):19-28.

10. Stojmirović B, Petrović D. Uticaj proteinurije na oštećenje tubulointersticijuma. Med Pregl. 2003;56:351-4.

11. Waller K, Ward K, Mahan J, Wismatt D. Current concepts in proteinuria. Clin Chem. 1989;3:755-65.

12. Carroll MF, Temte JL. Proteinuria in adults: a diagnostic approach. Am Fam Physician. 2000;62:1333-40.

13. Samal L, Linder JA. The primary care perspective on routine urine dipstick screening to identify patients with albuminuria. Clin J Am Soc Nephrol. 2013;8:131-5.

14. Ndamase S, Freercks R. The utility of urine sulphosalicylic acid testing in the detection of non-albumin proteinuria. SAMJ 2015;105:1.

15. Urine protein sulfosalycil acid precipitation test (SSA). Available from: http://www.austincc.edu/mlt/ua/UrineProteinSulfosalicylicAcidPrecipitationTest.pdf

16. Delanghe J. New screening diagnostic techniques in urinalysis. Acta Clin Belg. 2007;62:155-61.

17. National Kidney Foundation. KDOQI Guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(Suppl 1):1-266.

18. White SL, Yu R, Craig JC, Polkinghorne KR, Atkins RC, Chadban SJ. Diagnostic accuracy of urine dipsticks for detection of albuminuria in the general community. Am J Kidney Dis. 2011;58:19-28.

19. Delanghe J, Speeckaert M. Preanalytical requirements of urinalysis. Biochem Med. 2014;24:89-104.

Rad je primljen 16. VII 2015.
Recenziran 16. IX 2015.
Prihvaćen za štampu 23. XI 2015.
BIBLID.0025-8105:(2016):LXIX:7-8:197-202.

University of Banja Luka, Republic of Srpska
Faculty of Medicine

Original study
Originalni naučni rad
UDK 616.314-089.28/.29:613
DOI: 10.2298/MPNS1608203G

IMPACT OF DENTURES ON ORAL HEALTH-RELATED QUALITY OF LIFE

UTICAJ STOMATOPROTETIČKIH NADOKNADA NA KVALITET DENTALNOG ZDRAVLJA

Sanja GNJATO

Summary

Introduction. Stomatoprothetic dentures are one of the factors of oral health and life quality of people of all ages. The aim of the paper is to make a connection between the oral health quality and quality of life on one side and the type of denture on the other. **Material and Methods.** This research study was conducted on the sample of 360 patients singled out in three numerically identical groups as follows: group I – patients treated with fixed dentures, group II - patients treated with mobile dentures, and group III – patients treated with both fixed and mobile dentures. The oral health quality was observed via five parameters: anamnestic data, symptoms of ill-functioning of basic functions in stomatogenic system (chewing and speech), extra oral examination, intraoral examination, and dental abilities. For some oral health quality parameters, the index of quality was determined. **Results**. Analyses of our three target groups of patients indicated that the patients from group I (treated with fixed dentures) suffered the least negative effects in line with the observed parameters; they are followed by patients from group III (combined dentures) and patients from group II (mobile dentures), respectively. **Conclusion.** Our research study showed that some oral health parameters have different impacts on health and life quality in patients treated with different stomatoprosthetic dentures.

Key words: Oral Health; Dental Care; Quality of Life; Denture, Partial, Fixed; Denture, Partial, Removable; Dental Records; Patient Satisfaction

Sažetak

Uvod. Stomatoprotetičke nadoknade su jedan od faktora dentalnog zdravlja i kvaliteta života čoveka svih životnih doba. Cilj rada je da se kvalitet dentalnog zdravlja i kvaliteta života pacijenata dovede u vezu s vrstom stomatoprotetičke nadoknade. **Materijal i metode.** Istraživanje je sprovedeno na uzorku od 360 pacijenata, svrstanih u tri brojčano jednake grupe: I grupa – pacijenti sa fiksnim stomatoprotetičkim nadoknadama; II grupa – pacijenti sa mobilnim stomatoprotetičkim nadoknadama; III grupa – pacijenti sa mobilnim i fiksnim stomatoprotetičkim nadoknadama. Kvalitet dentalnog zdravlja posmatran je kroz pet dimenzija: anamnestički podaci, simptomi poremećaja osnovnih funkcija stomatogenog sistema (žvakanje i govor), ekstraoralni pregled, intraoralni pregled i dentalne sposobnosti. Za pojedine dimenzije kvaliteta dentalnog zdravlja određen je indeks kvaliteta. **Rezultati**. Analize u odnosu na istraživačke grupe pokazuju da pacijenti I grupe (sa fiksnim stomatoprotetičkim nadoknadama) imaju najmanje negativnih uticaja u odnosu na ispitivana obeležja, slede pacijenti III grupe (sa kombinovanim stomatoprotetičkim nadoknadama), dok su na trećem mestu pacijenti II grupe (sa mobilnim stomatoprotetičkim nadoknadama). **Zaključak.** Istraživanje je pokazalo da pojedine dimenzije dentalnog zdravlja različito utiču na kvalitet dentalnog zdravlja i kvalitet života pacijenata sa različitim stomatoprotetičkim nadoknadama.

Ključne reči: oralno zdravlje; stomatološka zaštita; kvalitet života; parcijalne fiksne proteze; parcijalne mobilne proteze; zubni kartoni; zadovoljstvo pacijenta

Introduction

One of the most important goals of dental care and protection is to help patients preserve their oral health and become satisfied with their oral healthcare and quality of life in general [1–6]. Generally, dental health is still not being paid enough attention as it does not directly endanger a patient's life. There are also researchers who still ignore the effects that the oral cavity and teeth have on general health condition [7]. Still, research studies on dental and oral health and a whole range of factors that affect quality of life (QoL – Quality of Life) have significantly advanced in late 20th and early 21st century [8–13].

Dental status and problems with stomatoprosthetic rehabilitation affect social activities such as work ability, family and parenting actions, emotional life, etc. Stomatognathic issues, also recognized as patients' satisfaction with their dental status, are evident through esthetics, performance and function [14–18].

Some studies [19–22] suggest that dental diseases and consequences of stomatoprosthetic treatment affect patients' ability to enjoy life, interact with other people, succeed at work and remain positive. There are different indicators of patients' dental status satisfaction such as chewing, taste sense, pain, speech ability, esthetic sensation etc. All these affect the Quality of Life (QoL) [24–27].

Corresponding Author: Doc. dr Sanja Gnjato, Univerzitet u Banja Luci, Medicinski fakultet, 78000 Banja Luka, Save Mrkalja 14, Republika Srpska, E-mail: sanja.gnjato@gmail.com

Speaking of the effect of oral health on the quality of life, many authors infer that patients treated with mobile dentures are more satisfied in comparison with those patients treated with fixed dentures [28, 29]. Some studies showed that oral health largelly affected patients' quality of life so that many patients observed the negative impact of the impaired dental health on their psychological, social and physical aspects of life [30].

According to some studies, the psychological profile, regardless of age and sex, largely affects the subjective idea of quality of life in line with stomatoprosthetic treatment [31]. Some clinical studies [32, 33] particularly [34–37] investigated the quality of oral health of patients who had undergone stomatoprosthetic treatment and the general conclusion was that dental implants resulted in short-term quality improvement when compared to conventional treatments. Furthermore, there are studies which inferred that functional limitations and restricted social functions largely affected the quality of life [38, 39]. Some studies on oral health proved that diet affected the dental caries prevention [40] as well as the knowledge and dental behavior of parents and school children [41]. Other studies on oral status and quality of oral health [42, 43] pointed out the complexity of this issue and proved that there was no universal approach to its solution.

Material and Methods

This study included three groups of patients treated with different types of dentures. There were 180 male and 180 females patients. The following three age groups were made: a) up to 60 years of age, b) between the ages of 60 and 70 years and c) over 70 years of age. Each age groups included 120 patients, which made the total sample of 360 patients.

I – The first group of patients were those treated with fixed dentures, i.e. 120 patients. This groups consisted of patients treated with fixed dentures covering at least 50% of both maxillary and mandibular teeth;

II – The second group consisted of patients treated with mobile dentures, i.e. 120 patients. Among these patients, 30 were treated with both total dentures, 30 were treated with total maxillary and partial mandibular dentures, 30 patients were treated with partial maxillary and total mandibular dentures, and 30 patients were treated with partial mandibular and maxillary dentures.

III – The third group of patients were those treated with both mobile and fixed dentures, i.e. 120 patients who met the following conditions: a) total maxillary denture and fixed mandibular denture – 30 patients, b) total mandibular denture and fixed maxillary denture – 30 patients, c) combined fixed and mobile maxillary dentures and partial mandibular dentures – 30 patients, d) combined fixed and mobile mandibular dentures and partial maxillary dentures – 30 patients.

A special dental chart was designed for the purpose of our study and data were recorded for each patient individually. The following data based upon 94 features were entered into the chart:

a) General information: ordinal number/group/protocole number; personal identification number; surname (name of one parent) and name of the patient; address; telephone number; sex; birth year; date of examination;

a) Anamnestic data: 16 features;

b) Symptoms of ill-functioning of stomatogenic system (speech and chewing): 6 features;

c) Clinical examination – dental status: extraoral examination: 8 features;

d) Clinical examination – dental status: intraoral examination: 12 features;

e) Additional clinical procedures: 5 features;

f) Imaging of temporomandibular joints: 3 features;

g) Dental ability questionnaire: 36 features into 5 subgroups.

The patients had been monitored for 17 months, from May 2012 to September 2013. Clinical dental status, dental abilities, and psychological profile of pateints were observed at the beginning of the reasearch, after six months and at the end.

The quality of oral health was observed through the following 5 dimension:

– Anamnestic data

– Symptoms of ill-functioning basic functions of stomatogenic system (chewing and speech)

– Extraoral examination

– Intraoral examination

– Dental abilities

The oral health quality index was determined by the linear regression model with the hierarchical barycenter coefficients, assuming that the adequate regression residues were equal to zero [44]. Thus, the oral health quality index was introduced through the general regression equation:

$$I_{KDZ}=K_A*I_A+K_S*I_S+K_{DSE}*I_{DSE}+K_{DSI}*I_{DSI}+K_{DS}*I_{DS}$$

I_{KDZ} – dependent variable: index (score) of oral life quality,

K_A – regression coefficient: hierarchical barycenter coefficient for the "anamnestic" category of oral health quality,

I_A – independent variable: index (score) of the "anamnestic" category of oral health quality,

K_S – regression coefficient: hierarchical barycenter coefficient for the „symptoms" category of oral health quality,

I_S – independent variable: index (score) of the "symptoms" category of oral health quality,

K_{DSE} – regression coefficient: hierarchical barycenter coefficient for the „extraoral dental status" category of oral health quality,

I_{DSE} – independent variable: index (score) of the "extra-oral dental status" category of oral health quality,

K_{DSI} – regression coefficient: hierarchical barycenter coefficient for the "intraoral dental status" category of oral health quality,

I_{DSI} – independent variable: index (score) of the "intraoral dental status" category of oral health quality,

K_{DS} – regression coefficient: hierarchical barycenter coefficient for the "dental abilities" category of oral health quality,

I_{DS} – independent variable: index (score) of the "dental abilities" category of oral health quality.

In order to determine the oral health quality index, it was necessary to set the following indicators for each observed category:

a) relevance ranking,

b) relevance level – barycenter coefficient: hierarchical, uniformed, etc.,

c) contribution: positive – the more the better, negative – the less the worse.

In this regard, hierarchical barycenter coefficient for each category was determined and the ranking hierarchy read "the strongest impact, the highest coefficient; the weakest impact, the lowest coefficient".

– The first impact category (rank one) is the "quality index of intraoral examination";

– The second impact category (rank two) is the "quality index of extraoral examination";

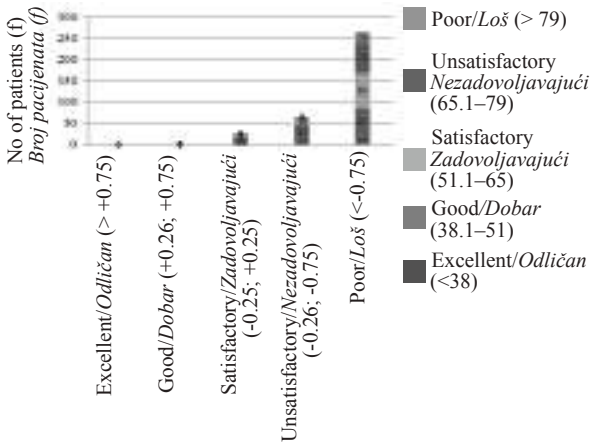
– The third category (rank three) is the "quality index of ill-functioning of basic functions (chewing and speech)"

– The fourth category (rank two) is the "quality index of anamnesis";

– The fifth category (rank two) is the "quality index of dental abilities".

An algorithm of the oral health quality index was provided based on the general regression equation and **Table 1**:

$$I_{KDZ} = 2 * I_A + 3 * I_S + 4 * I_{DSE} + 5I_{DSI} + 1 * I_{DS}$$



Graph 1. Distribution of patients per oral health quality in reference to the "appearance" category of dental abilities

Grafikon 1. *Distribucija pacijenata po kvalitetu dentalnog zdravlja u odnosu na dimenziju dentalnih sposobnosti „Izgled"*

Testing has shown that there are highly statistically relevant differences between the oral health quality and the "appearance" category of dental abilities (p=0.0029).

The following step in calculating the Oral health quality index was to set an algorithm for specific categories of oral health quality and their components.

The procedure was as follows:

a) Oral health quality category "Anamnesis".

The equation for calculation of the "Anamnesis" category of oral health quality was:

$$IA = \frac{1}{13}(4*KOM + 3*FR + 13*OT + 12*TMS + 10*OKT + 9*$$

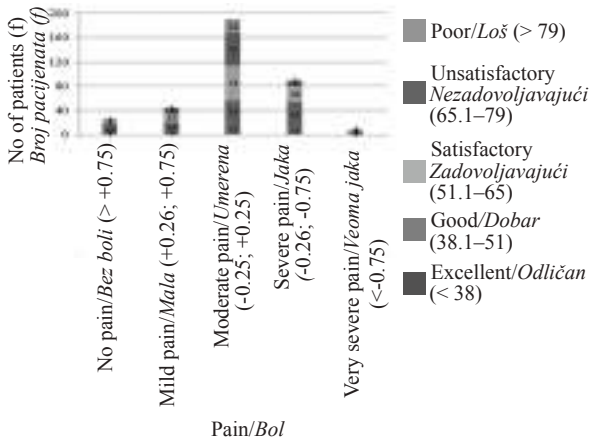
$$OKD + 6*MZ + 11*BDP + 8*PAR + 5*\check{S} + 7*AT + 1*SP + 2*NN)$$

b) "Symptoms" category of oral health quality.

Table 1. Values of hierarchical barycentre coefficients of different dimensions of oral health quality

Tabela 1*. Veličine hijerarhijskih težinskih koeficijenata pojedinih dimenzija kvaliteta dentalnog zdravlja*

Independent variables of oral health quality *Nezavisne varijable kvaliteta dentalnog zdravlja*	Quality/*Kvalitet*		
	Relevance ranking *Rang značaja*	Contribution *Doprinos* (+,-)	Relevance level – barycentre coefficient *Nivo značaja – težinski koeficijent*
Quality index of anamnesis *Indeks kvaliteta anamneze*	4	+	2
Quality index of symptoms of ill-functioning basic functions (chewing and speech) *Indeks kvaliteta simptoma poremećaja osnovnih funkcija (žvakanje i govor)*	3	+	3
Quality index of extraoral examination *Indeks kvaliteta ekstraortalnog pregleda*	2	+	4
Quality index of intra-oral examination *Indeks kvaliteta nog pregleda*	1	+	5
Quality index of dental abilities *Indeks kvaliteta dentalnih sposobnosti*	5	+	1



Graph 2. Distribution of patients per oral health quality in reference to the "pain" category of dental abilities
Grafikon 2. *Distribucija pacijenata po kvalitetu dentalnog zdravlja u odnosu na dimenziju dentalnih sposobnosti „Bol"*
Testing has shown that there are highly statistically relevant differences between the oral health quality and the "pain" category of dental abilities (p=0.0000).

The equation for calculation of the "Symptoms" category of oral health quality was:

$$IS = \frac{1}{6}(6 * I_S + 1 * II_S + 2 * III_S + 4 * IV_S + 5 * V_S + 3 * VI_S)$$

c) "Extraoral dental status" category of oral health quality.

The equation for calculation of the "Extraoral dental status" category of oral health quality was:

$$IDSE = \frac{1}{8}(3 * AL + 2 * DZ + 8 * NV + 1 *$$

$$JG + 7 * PZ + 6 * POS + 5 * KZ + 4 * POMM)$$

d) "Intraoral dental status" category of oral health quality.

The equation for calculation of the "Intraoral dental status" category of oral health quality was:

$$IDSI = \frac{1}{11}(4 * VZ + 7 * SP + 2 * NH + 9 * PBS + 8 * PPT +$$

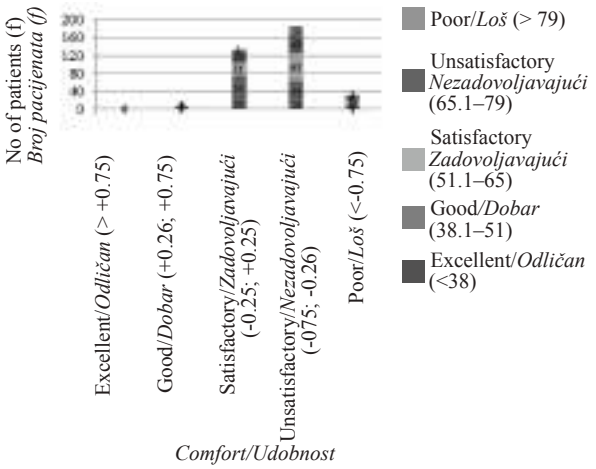
$$11 * OAG + 10 * PKI + 6 * J + 3 * VPUD + 5 * VP + 1 * SSN)$$

e) "Dental abilities" category of oral health quality.

The equation for calculation of the "Dental abilities" category of oral health quality was:

$$I_{DS} = \frac{1}{5}(-1 * IZ + 5 * BOL + 3 * UD + 2 * IS - 4 * OJ)$$

The obtained values of different categories of oral health quality are relative as they depend on the obtained result, which means that another study might provide different results. It would be more reliable if there were standards for oral health quality with absolute value categories. However, even such a relative assessment of oral health quality is quite valid and meets the needs of initial studies within this field. After multiple studies and polycentric research within the field of oral health quality, we may expect the method of determination of oral health quality to become widely applied.



Graph 3. Distribution of patients per oral health quality in reference with the "comfort" category of dental abilities
Grafikon 3. *Distribucija pacijenata po grupama u kvalitetu dentalnog zdravlja na dimenziju dentalnih sposobnosti „Udobnost"*
Testing has shown that there are no statistically relevant differences between the oral health quality and the "comfort" category of dental abilities (p=0.0633).

Results

For the purpose of this paper, only partial results are provided in order to introduce a standardized model for monitoring dental abilities and oral health quality in clinical practice as an interaction between the practitioner and patient. We provided a detailed outline of the following features:

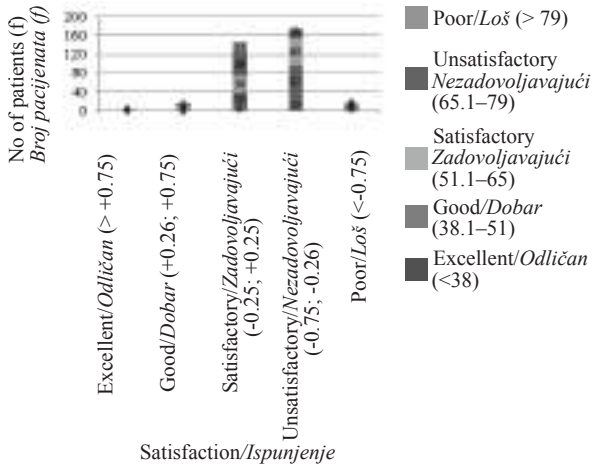
– Demographic characteristics (9 features);

– Anamnestic data (16 features): cause of visit, comorbidity, risk factors, basic symptoms, pain in masticatory system, occlusal trauma, occlusal disharmony, tooth mobility, toothache, parodontopathy, teeth grinding, arthropatic pain, time elapsed between a tooth loss to getting dentures, mobile or fixed denture treatment, the denture stablility, denture discomfort;

– Symptoms of ill-functioning stomatogenic system (chewing and speech) (6 features): any chewing discomfort, food-chewing ability, avoiding food, unilateral or bilateral mastication, discomfort during or after chewing, sound produced during the chewing;

– Dental status – Extraoral (8 features): face asymmetry caused by dental status, tooth visibility during speech production, unequal jaws, speech clarity, joint mobility, palpatory sensitivity of joints, joint crepitation, palpatory sensitivity of masticatory muscles;

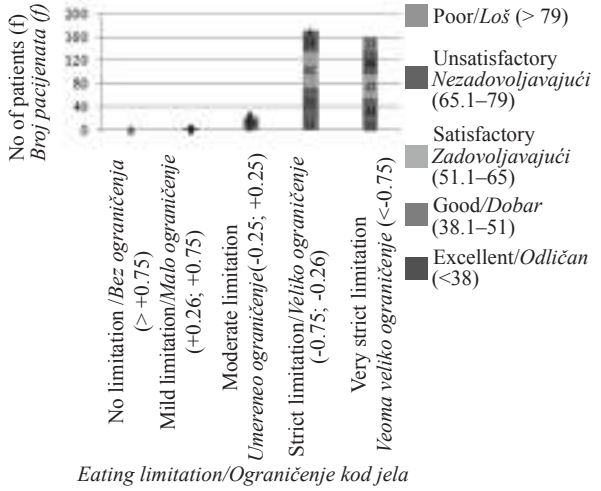
– Dental status – Intraoral (12 features): tooth status, tooth vitality, parodontium condition, hygi-



Graph 4. Distribution of patients per oral health quality in reference to the „satisfaction" category of dental abilities

Grafikon 4. Distribucija pacijenata po kvalitetu dentalnog zdravlja u odnosu na dimenziju dentalnih sposobnosti „Ispunjenje"

Testing has shown that there are highly statistically relevant differences between the oral health quality and the "satisfaction" category of dental abilities (p=0.0000).



Graph 5. Distribution of patients per oral health quality in reference to the "eating limitation" category of dental abilities

Grafikon 5. *Distribucija pacijenata po kvalitetu dentalnog zdravlja u odnosu na dimenziju dentalnih sposobnosti „Ograničenja kod jela"*

Testing has shown that there are no statistically relevant differences between the oral health quality and the "eating limitation" category of dental abilities (p=0.0633).

ene level, mucous change in the membrane color, pathological changes in all tissues, the shape of alveolar ridge, bone salience in jaws, tongue status and characteristics, height of mouth cavity floor, saliva viscosity, condition of old denture;

– Additional clinical data (8 features): retro-alveolar screening, orthopantomographic screening, models for studies, electromyographic screening, joint artography, joint tomography, joint magnetic resonance imaging;

– Dental abilities with five categories: appearance (4 features), pain (4 features), comfort (7 features), performance (15 features), eating limitations (6 features);

The quality of oral health was calculated based upon the five groups of features (anamnesis, symptoms, extraoral status, intraoral status and dental abilities) marked as five categories (poor, unsatisfactory, satisfactory, good, excellent).

Results of analysis per research groups in line with basic dimensions of dental abilities

While performing a comparative analysis of the sample regarding dental abilities, we used the total sample. For the purpose of the paper, a brief version of comparative analysis of the sample in line with dental abilities is provided.

Our study showed that patients from all three groups were unsatisfied with the dental ability *"Appearance"* regarding the tooth appearance, distribution and color and there were statistically significant differences among the groups (p=0.0000).

Irrespective of age, all three groups of patients felt different intensity for the dental ability *"Pain"*. Testings proved that there were statistically significant differences among the groups (p=0,0000).

In line with dental ability *"Commodity"*, patients were unsatisfied with both teeth and gums and testings proved that there were statistically significant differences among the groups (p=0,0000).

In line with dental ability *"Satisfaction"*, most patients in all three groups made a connection between tooth appearance and their everyday activities and testings proved that there were statistically significant differences among the groups (p=0.0000).

In line with dental ability *"Eating limitations",* all three groups of patients felt different intensity. Testings proved that there were statistically significant differences among the groups (p=0.0000).

The study showed that total dental abilities in patients from all three groups were mostly unsatisfactory and only few patients were either fully or poorly satisfied. There were statistically highly significant differences among groups in line with total dental abilities (p=0.0000).

Results of analysis of the quality of oral health

While performing a comparative analysis of the sample in reference among dental abilities, we used the total sample. For the purpose of the paper, a brief version is provided.

Testing showed that there were highly statistically relevant differences between the oral health quality and the "appearance" category of dental abilities (p=0.0029) **(Graph 1)**.

Testing has shown that there were highly statistically relevant differences between the oral health quality and the „pain" category of dental abilities (p=0.0000) **(Graph 2)**.

Testing showed that there were no statistically relevant differences between the oral health quality and the „commodity" category of dental abilities (p=0.0633) **(Graph 3).**

Testing showed that there were highly statistically relevant differences between the oral health quality and the „satisfaction" category of dental abilities (p=0.0000) **(Graph 4).**

Testing showed that there were no statistically relevant differences between the oral health quality and the „eating limitation" category of dental abilities (p=0.0633) **(Graph 5).**

In addition no statistically relevant differences were found between the oral health quality and total dental ability (dental ability score) **(p=0.0797).**

Discussion

The research results presented in this study suggest that different levels of oral health and patients' satisfaction and quality of life depend on a whole range of factors. In this regard, dentures have the greatest impact. Different studies [45], including the one presented hereby [46], have inferred that a patient's satisfaction with oral health, apart from objective reasons, also depends on subjective reasons, i.e. one's psychological profile.

In addition, results of some studies confirmed a connection between the oral health and physical, mental, social and general health condition and found a link between the oral health index and index of perception of general health (as well as a connection between the mental health index and two indicators of phycisal health condition but to a lesser extent).

A special dental chart was designed and individual records were kept for each patient for the purpose of our study. The patients were asked to choose one of the following answers in the questionnaire on dental abilities: positive, neutral, and negative. Based on the "dental chart" data, a model of oral health quality was designed (a linear regression model with a hierarchical barycenter coefficient). Based on the model of quality of oral health, the index of oral health quality was calculated [46].

The study study sample was divided into three study groups. The first study group (patients treated with fixed dentures) had no subgroups, but the second and third groups (patients treated with mobile and combined dentures) were further divided into subgroups in order to estimate differences in oral health quality and quality of life. The analyses showed that 27 study features had statistical and high statistical relevance, and 4 features had no statistical relevance.

Furthermore, analyses showed that the patients treated with fixed dentures had the least negative effects of the tested features, followed by the patients treated with mobile and fixed dentures, and finally the patients treated with mobile dentures.

The analyses of the oral health quality indicated that 36 out of 49 analyzed features had significant or highly significant relevance, whereas 7 features had no relevance. In addition, 6 features did not meet all the conditions necessary for the testing of statistical relevance. All the target features with statistically relevant differences in reference to oral health were proved to have a negative affect on dental abilities, and the patients in higher categories of oral health were less influenced by the negative target features.

Hence, the results of comparative analyses of target features and oral health quality for statistically relevant differences have shown that the oral health quality is in accordance with the age and denture type and depends on the following: basic difficulties, IKP contact, tooth mobility, paradontopathy, parafunction activities of OF system (bruxism), arthropathy, time elapsed from tooth edentulation to denture embedding, denture stability, comfort/discomfort in wearing dentures, etc. Oral health quality is highly influenced by mastication followed by sound effects, face asymmetry, tooth length, vertical dimension of occlusion, speech clarity, mobility and palpatory sensitivity of temporomandibular joints, crepitation in temporomandibular joints, palpatory sensitivity of masticatory muscles, condition of paradonths, hygiene level, condition of mouth cavity mucous membrane, pathological changes in mouth cavity tissue, shape of a toothless alveolar ridge, bony pops in the jaw, the tongue shape, size and function, saliva viscosity, condition of old dentures. Taking into account the "appearance" category of dental abilities, a patient's oral health quality is affected by looks, colour, and tooth distribution. As for the "pain" category of dental abilities, oral life quality is affected by the pain intensity and duration, whereas "satisfaction" is affected by the tooth appearance.

The comparative analyses of the target features and oral health quality in reference to the study groups (in case of statistically relevant differences) showed that oral health quality was highly affected by dental pain (caries, pulpitis, paradontopathy, occlusal trauma), undefined pain, food avoidance, pain due to inadequate denture, vomiting urge, difficulty in swallowing, condition of retention teeth and their supporting tissue, position of the remaining teeth, contact between the neighboring teeth and antagonist, and the extent of absorption of the residual alveolar ridge.

Results of comparative analysis of the target features and oral health quality for statistically insignificant differences showed that oral health quality was not affected by sex and age of patients.

Conclusion

This paper estimated a relation among the dental status, quality of oral health and quality of life of patients treated with different stomatoprosthetic dentures (mobile, fixed, combined).

The results of this research study have shown that are no statistically significant differences in line with gender in comparison with the relevant research parameters; there are statistically significant and highly significant differences between age groups in line with "pain", "satisfaction" and "eating limitation" dimensions of dental abilities in comparison with "appearance" and "commodity" dimensions.

Analyses of study groups also indicated that the patients from group I (fixed dentures) suffered less negative impact in line with the referential features; the patients from group III came second (combined dentures); the patients from group II were third (mobile dentures).

Apendix I
Elements for calculation of quality of oral health

Equation for calculation of the index of oral health quality

$$I_{KDZ} = 2_* I_A + 3_* I_S + 4_* I_{DSE} + 5_* I_{DSI} + 1_* I_{DS}$$

KDZ- Oral life quality
I_A- index (score) of the "anamnestic" category of oral health quality
I_S- Index of quality of symptoms of ill-functioning basic functions
I_{DSE}- index (score) of the "extra-oral dental status" category of oral health quality
I_{DSI}- index (score) of the "intraoral dental status" category of oral health quality
I_{DS}- index (score) of the "dental abilities" category of oral health quality.

Equation for calculation of index of "anamnesis" dimension of oral health quality

$$IA = \frac{1}{13}(4\,KOM + 3 * FR + 13 * OT + 12 * TMS + 10 * OKT + 9 * OKD + 6 * MZ * + 11 * BDP + 8 * PAR + 5 * \check{S} + 7 * AT + 1 * SP + 2 * NN)$$

KOM - commorbidity; FR - factors of risk; OT - basic problems; TMS - masticatory system problems; OKT - occlusal trauma; OKD - occlusal disharmony; MZ - tooth mobility; BPD - dental pain; PAR - Parodontopathy; Š - bruxism; AT - Arthropatic problems; SP - denture stability; NN - discomfort with wearing dentures.

Equation for calculation of index of "symptom" dimension of oral health quality

$$IS = \frac{1}{6}(6 * Is + 1 * II_S + 2 * III_S + 4 * IV_S + 5 * V_S + 3 * VI_S)$$

I_S – Does the patient have chewing difficulties?
II_S – Can the patient chew food?
III_S – Does the patient avoid food?
IV_S – Does the patient use both or one side of the jaw when chewing food?
V_S – Does the patient feel discomfort during or after chewing?
VI_S – Is chewing accompanied with a sound effect?

Equation for calculation of index of "dental status extraoral" dimension of oral health quality

$$IDSE = \frac{1}{8}\frac{1}{8}(3 * AL + 2 * DZ + 8 * NV + 1 * JG + 7 * PZ + 6 * POS + 5 * KZ + 4 * POMM)$$

AL - face assymetry; DZ - tooth length; NV - jaw disproportion; JG - speech clarity; PZ - joint mobility; POS - Palpatory joint sensitivity; KZ - joint crepitation; POMM - Palpatory sensitivity of masticatory muscles.

Equation for calculation of index of "dental status intraoral" dimension of oral health quality

$$IDSI = \frac{1}{11}(4 * VZ + 7 * SP + 2 * NH + 9 * PBS + 8 * PPT + 11 * OAG + 10 * PKI + 6 * J + 3 * VPUD + 5 * VP + 1 * SSN)$$

VZ - tooth vitality; SP - paradonthium condition; NH - level of higyene; PBS - muscuos membrane discoloration; PPT - Pathological changes in all tissue; OAG - alveolar ridge shape; PKI - bone salience in lower and upper jaws; J - shape, size and function of the tongue; VPUD - heigth of oral cavity floor; VP - saliva viscosity; SSN - old denture condition.

Equation for calculation of index of "dental abilities" dimension of oral health quality

$$I_{DS} = \frac{11}{55}(-1 * IZ + 5 * BOL + 3 * UD + 2 * IS - 4 * OJ)$$

IZ – Dimension: APPEARANCE
BOL– Dimension: PAIN
UD – Dimension: COMFORT
IS – Dimension: SATISFACTION
OJ – Dimension: EATING LIMITATIONS

References

1. Džambas LD, Bokor-Bratić M, Džambas B, Selaković S, Hrvaćanin SO, Jefić B. Aktivan i kvalitetan život starih osoba rehabilitovanih totalnim zubnim protezama. Gerontologija. 2002;30(1):191-5.

2. Džambas Lj, Čupić S. Kvalitet života pacijenata saniranih postresekcionim intraoralnom protezom nakon hirurškog odstranjenja malignih tumora gornje vilice. Medicina danas. 2005;4(3-4):458-62.

3. Džambas Lj, Dželetović-Milošević I, Vukadinov J, Hrvaćanin S. Activee and high quality life of eldery individuals rehabilitated with removable and fixed dental prosthetics. 2-nd Croatian gerontological congress with international participation. The journal of the croatian medical association, March 912, 2006; Opatija, Croatia, 143.

4. Džambas Lj, Jeremić Knežević M, Anđelković A, Vukić Z. Kvalitet života starih osoba saniranih totalnim imedijalnim zubnim protezama. Osmi gerontološki Kongres sa međunarodnim učešćem. Knjiga rezimea, Vrnjačka Banja; maj 2010. str. 235-6.

5. Džambas Lj, Gnjato S, Vukić Z. Life Quality of the eldery with immediate total denture. Arch Biol Sci. 2012;64:1141-5.

6. Al-Omiri MK, Karasneh J. Relationship between oral health-related quality of life, satisfaction and personality in patients with prosthetic rehabilitations. J Prosthodontics. 2010;19(1):2-9.

7. Steele JG, Ayatouahi SM, Walls AW, et al. Clinical factors related to reported satisfaction with oral function amongst dentate older adults in England. Community Dent Oral Epidemiol. 1997;25:143-9.

8. Awad MA, Locker D, Korner-Bitensky N, Feine JS. Measuring the effect of intra-oral implant rehabilitation on health-related quality of life in a randomized controlled clinical trial. J Dent Res. 2000;79:1659-63.

9. Bae KH, Kim C, Paik DI, Kim JB. A comparison of oral health related quality of life between complete and partial removable denture-wearing older adults in Korea. J Oral Rehabil. 2006;33(5):317-22.

10. Bae KH, Kim HD, Jung SH, Park DY, Kim JB, Paik DI, et al. Validation of the Korean version of the oral health impact profile among the Korean elderly. Community Dent Oral Epidemiol. 2007;35(1):73-9.

11. Broder HL, Wilson-Genderson M, Sischo L. Examination of a theoretical model for oral health-related quality of life among youths with cleft. Am J Public Health. 2014;104(5):865-71.

12. Cohen LK. The emerging field of oral health-related quality of life outcomes research. In: Slade GD, editor. Measuring oral health and quality of life. Chapel Hill: University of North Carolina; 1997.

13. Ellis JS, Pelekis ND, Thomason MJ. Conventional rehabilitation of edentulous patients, the impact on oral health-related quality of life and patient satisfaction. J Prosthodont. 2007;16(1):37-42.

14. Leao A, Sheiham A. The development of a socio-dental measure of dental impacts on daily living. Community Dent Health. 1996;13(1):22-6.

15. Leao A, Sheiham A. Relation between clinical dental status and subjective impacts on daily living. J Dent Res. 1995; 74:1408-13.

16. Al-Omiri MK, Abu Hantash RO, Abu Yunis M, Lynch L. Relationship between personality and impacts of implant treatment on daily living. Clin Implant Dent Relat Res. 2012;14 Suppl 1:e2-10.

17. Al-Omiri MK, Sghaircen MG, Al-Qudah AA, Abu Hannmad O, Lynch CD, Lynch E. Relationship between impacts of removable prosthodontic rehabilitation on daily living, satisfaction and personality profiles. J Dent. 2014;42(3):366-72.

18. Reisine S. Dental health and public policy: the social impact of dental disease. Am J Public Health. 1985;74:27-30.

19. Strauss R, Hunt R. Understanding the value of teeth to older adults: influences on the quality of life. J Am Dent Ass. 1993;124:105-10.

20. Torres BL, Costa FO, Modena CM, Cota LO, Côrtes MI, Seraidarian PI. Association between personality traits and quality of life in patients treated with conventional mandibular dentures or implant-supported overdentures. J Oral Rehabil. 2011;38(6):454-61.

21. Thomason JM, Heydeeke G, Feine JS, Ellis JS. How do patients perceive the benefit of reconstructive dentistry with regard to oral health-related quality of life and patient satisfaction? A systematic review. Clin Oral Implants Res. 2007;18 Suppl 3:168-88.

22. Wright WG, Jones JA, Spiro A III, Rich SE, Kressin NR. Use of patient self-report oral health outcome measures in assessment of dental treatment outcomes. J Public Health Dent. 2009; 69(2):95-103.

23. Adulyanon S, Sheiham A, Oral Impacts on Daily Performances. In: Slade G, editor. Measuring Oral Health and Quality of Life. Chapel Hill: University of North Carolina; Dental Ecology; 1997.

24. Locker D, Slade GD. Oral health and quality of life among older adults: the oral health impact profile. J Can Dent Ass. 1993;59:830-44.

25. Michaud PL, de Grandmont P, Feine JS, Emami E. Measuring patient-based outcomes: Is treatment satisfaction associated with oral health-related quality of life? J Dent. 2012;40(8):624-31.

26. Ostberg AL, Hall-Lord ML. Oral health-related quality of life in older Swedish people with pain problems. Scandinavian Journal of Caring Sciences, 2011;25(3):510-6.

27. Perišić S, Palac A, Bunjevac T, Čelebić A. Development of a new chewing function questionnaire for assessment of a self-perceived chewing function. Community Dent Oral Epidemiol. 2013;41(6):565-73.

28. Reisine S, Locker D. Social, psychological and economic impacts of oral conditions and treatments. In: Cohen LK, Gift HC, editors. Disease prevention and oral health promotion. 1st ed. Copenhagen: Munksgaard; 1995. p. 33-72.

29. Ozhayat EB, Gotfredsen K. Effect of treatment with fixed and removable dental prostheses. An oral health-related quality of life study. J Oral Rehabil. 2012;39(1):28-36.

30. Ozhayat EB, Gotfredsen K, Elverdam B, Owall B. Comparison of an individual systematic interview method and the oral health impact profile. Responsiveness and ability of describing treatment effect of oral rehabilitation. J Oral Rehabil. 2010;37(8):604-14.

31. Needleman I, McGrath C, Floyd P, Biddle A. Impact of oral health on the life quality of periodontal patients. J Clin Periodontol. 2004;31(6):454-7.

32. Mattila AK, Pohjola V, Suominen AL, Joukamaa M, Lahti S. Difficulties in emotional regulation: association with poorer oral health-related quality of life in the general population. Eur J Oral Sci. 2012;120:3.

33. Llewellyn CD, Warnakulasuriya S. The impact of stomatological disease on oral health-related quality of life. Eur J Oral Sci. 2003;111(4):297-304.

34. Locker D. The burden of oral disorders in a population of older adults. Community Dental Health. 1992;9:109-24.

35. Locker D, Slade GD. Association between clinical and subjective indicators of oral health status in an older adult population, Gerodontology. 1994;11:108-14.

36. Locker D, Miller Y. Evaluation of subjective oral health status indicators, J. Public Health Dent. 1994;54:167-76.

37. Locker D, Jokovic A. Using subjective oral health status indicators to screen for dental care in older adults. Community Dent Oral Epidemiol. 1996;24:398-402.

38. Lee YH, Lee SK. The effect of oral care program for the elderly women of the visiting oral health care. Journal of Korean society of Dental Hygiene. 2012;12(2)365.

39. Kelly M, Steele J, Nuttall N, Bradnock G, Morris J, Nunn J, et al. Adult dental health survey: oral health in the United Kingdom, London. 1998.

40. Jevtić M, Pantelinac J. The role of nutrition in caries prevention and maintenance of oral health during pregnancy. Med Pregl. 2015;68(11-12):387-93.

41. Lalić M, Aleksić E, Gajić M, Malešević Đ. Oral health related knowledge and health behaviour of parents and school children. Med Pregl. 2013;66(1-2):70-80.

42. Locker D. Issues in measuring change in self-perceived oral health status. Community Dent Oral Epidemiol. 1998;26:41-7.

43. Locker D, Berka E, Jokovic A, Tompson B. Does self-weighting of items enhance the performance of an oral health-related quality of life questionnaire? Community Dent Oral Epidemiol. 2007;35(1):35-43.

44. Tanjga R. Statistika u medicini. Banja Luka: Medicinski fakultet; 2004.

45. Gnjato S. Kvalitet života pacijenata saniranih fiksnim i mobilnim stomatoprotetičkim radovima [doktorska disertacija]. Banja Luka: Medicinski fakultet Univerziteta u Banjoj Luci; 2014.

46. Petrović Đ. Primena metoda merenja kvaliteta života kao ishoda medicinskog tretmana u stomatologiji. Zbornik radova ECPD međunarodnih letnjih škola. Beograd; 2013. s. 128-48.

Rad je primljen 25. II 2016.
Recenziran 21. III 2016.
Prihvaćen za štampu 24. III 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:203-211.

Medical Center Zaječar, Department of Interventional Cardiology[1]
University Hospital Niš, Department of Interventional Cardiology[2]
Medical Center Zaječar, Department of Nuclear Medicine[3]

Original study
Originalni naučni rad
UDK 616.124-08:615.817
DOI: 10.2298/MPNS1608212M

THE EFFECT OF RIGHT VENTRICULAR PACEMAKER LEAD POSITION ON FUNCTIONAL STATUS IN PATIENTS WITH PRESERVED LEFT VENTRICULAR EJECTION FRACTION

EFEKAT POLOŽAJA PEJSMEJKER ELEKTRODE U DESNOJ KOMORI NA FUNKCIONALNI STATUS PACIJENATA SA OČUVANOM EJEKCIONOM FRAKCIJOM LEVE KOMORE

Vladimir M. MITOV[1], Zoran PERIŠIĆ[2], Aleksandar JOLIĆ[1], Tomislav KOSTIĆ[2], Aleksandar ALEKSIĆ[1] and Željka ALEKSIĆ[3]

Summary

Introduction. The study was aimed at assessing the difference between the right ventricle apex versus the right ventricular outflow tract lead position in functional capacity in the patients with the preserved left ventricular ejection fraction after 12 months of pacemaker stimulation. **Material and Methods.** This was a prospective, randomized, follow-up study, which lasted for 12 months. The study sample included 132 consecutive patients who were implanted with permanent anti-bradicardiac pacemaker. Regarding the right ventricular lead position the patients were divided into two groups: the right ventricle apex group consisting of 61 patients with right ventricular apex lead position. The right ventricular outflow tract group included 71 patients with right ventricular outflow tract lead position. Functional capacity was assessed by Minnesota Living With Heart Failure score, New York Heart Association class and Six Minute Walk Test. Left ventricular ejection fraction was assessed by echocardiography. **Results.** Minnesota Living With Heart Failure score and New York Heart Association class had a statistically significant improvement in both study groups. The patients from right ventricle apex group walked 20.95% (p=0.03) more in comparison to starting values. The patients from right ventricular outflow tract group walked only 13.63% (p=0.09) longer distance than the starting one. **Conclusion.** Analysis of tests of functional status New York Heart Association class and Minnesota Living With Heart Failure questionnaire showed an even improvement in the right ventricle apex and right ventricular outflow tract groups. Analysis of 6 minute walk test showed that only the patients with the preserved left ventricular ejection fraction from the right ventricle apex group had a significant improvement after 12 months of pacemaker stimulation..

Key words*:* Pacemaker, Artificial; Electrodes, Implanted; Heart Ventricles; Ventricular Function, Left; Stroke Volume; Questionnaires; Walking; Bradycardia; Treatment Outcome

Sažetak

Uvod. Cilj istraživanja je procena uticaja stimulacije pejsmejkerom iz vrha desne komore (*Right Ventricle Apex*) i izlaznog trakta (*Right Ventricle Outflow Tract*) desne komore, nakon 12 meseci stimulacije pejsmejkerom, na funkcionalni status pacijenata sa očuvanom ejekcionom frakcijom leve komore. **Materijal i metode.** Sprovedena je prospektivna, randomizovana studija praćenja u trajanju od 12 meseci. Istraživanjem su obuhvaćena 132 pacijenta kod kojih je implantiran permanentni antibradikardni pejsmejker. U odnosu na položaj komorske elektrode, pacijenti su podeljeni u dve grupe: prva grupa – 61 pacijent sa elektrodom pozicioniranom u vrhu desne komore; druga grupa – 71 pacijent sa elektrodom pozicioniranom u izlaznom traktu desne komore. Funkcionalni status pacijenata procenjivan je preko upitnika *Minnesota Living With Heart Failure* i određivanjem vrednosti skora, određivanjem pripadnosti *New York Heart Association* klasi, kao i šestominutnim testom hodanja. Ejekciona frakcija leve komore procenjivana je ehokardiografskim pregledom. **Rezultati.** Vrednosti *Minnesota Living With Heart Failure* skora i *New York Heart Association* klase imali su statistički značajno poboljšanje u obe praćene grupe pacijenata. U prvoj grupi pacijenti su pelazili 20,95% (p = 0,03) duže distance u odnosu na početne vrednosti. U drugoj grupi pacijenti su pelazili samo 13,63% (p = 0,09) duže distance u odnosu na početne vrednosti. **Zaključak.** Testovi za procenu funkcionalnog statusa, *New York Heart Association* klasa i *Minnesota Living With Heart Failure* upitnik, pokazali su podjednako poboljšanje u obe grupe. Analizom rezultata šestominutnog testa hodanja dobijeno je da je statistički značajno funkcionalno poboljšanje imali samo pacijenti iz prve grupe, nakon 12 meseci stimulacije pejsmejkerom, kod pacijentata sa očuvanom ejekcionom frakcijom leve komore.

Ključne reči: pejsmejker; implantacija elektroda; srčane komore; funkcija leve komore srca; udarni volumen; upitnici; šetanje; bradikardija; ishod lečenja

Introduction

Standard pacemaker lead position and thus stimulation from right ventricle apex (RVA) is characterized by prolonging transseptal and intraventricular impulse conduction with QRS at least twice longer than the normal duration [1]. The pacemaker stimulation from right ventricular outflow tract (RVOT) provides stim-

Corresponding Author: Prim. dr sc. med. Vladimir Mitov, Medicinski centar Zaječar, Odeljenje invazivne kardiologije, 19000 Zaječar, Rasadnička bb, E-mail: mitov@ptt.rs

Abbreviations

RVA – right ventricle apex
RVOT – right ventricular outflow tract
MLWHF – Minnesota Living With Heart Failure
NYHA – New York Heart Association
6 MWT – Six Minute Walk Test
LVEF – left ventricular ejection fraction
VVIR – single chamber pacemaker
DDD – dual chamber pacemaker

ulus conduction that enables the chamber activation from the septum to the rest of the myocardium which in turn gives less dyssynchrony and shorter QRS duration [2–4]. Twenty years of experience and the results of multi centre randomized trials showed a benefit of alternative pacemaker stimulation site in the patients with a decreased left ventricular ejection fraction (LVEF), while the benefit is absent in the patients with preserved LVEF. However, the influence of lead position on the functional class, exercise capacity and quality of life in patients with preserved LVEF is still unclear [5]. Bacior et al. reviewed a number of studies involving patients with low LVEF, and concluded that almost all of the studies showed an improvement in the quality of life after pacemaker stimulation [6]. However, in the patients without a structural heart disease, Cano et al. found no difference in the quality of life, New York Heart Association (NYHA) class or physical endurance in RVOT versus RVA [7].

This study was aimed at assessing the difference between right ventricle apex versus right ventricular outflow tract lead position in functional capacity in patients with preserved left ventricular ejection fraction after 12 months of pacemaker stimulation.

Material and Methods

This was a prospective, randomized study, which lasted for 12 months and included 132 consecutive patients who were implanted with permanent antibradycardiac pacemaker in the Pacemaker Center-Medical Center Zaječar, Serbia in the period from 2010 to 2011. This study was approved by the Ethical Committee of Zajecar Health Center and by Faculty of Medicine of the University of Niš. All patients gave their informed consent to participate in this study. The following pacemakers were used: St. Jude Medical Verity ADx XL SR 5156 (USA), single chamber pacemaker (VVI), and Medtronic Sensia SEDR01 (USA) dual chamber pacemaker (DDD). Regarding the RV lead position the patients were divided into two groups: the RVOT group consisted of 71 patients with RV outflow tract lead position and the RVA group included 61 patients with RV apex lead position. Active fixation ventricular leads (St. Jude Medical Tendril 188TC/58) were used in the RVOT group and ventricular passive fixation leads (Medtronic 4074-58) were used in the RVA group. All patients with DDD pacemakers had a "J" passive fixation atrial lead Medtronic 4592-53.

The patients' functional capacity was assessed with 3 independent tests:

Minnesota Living With Heart Failure (MLWHF) questionnaire - which is filled by the patients by circling the number in front on the six degree scale of symptoms, from 0 to 5. The patient chooses 0 if the symptom is absent, or if the patient does not want to answer the question. Number 1 means that the symptom is mild, number 5 is for the most severe symptom. The questionnaire consists of 21 questions which depict the physical, mental, emotional, and social status of the patient. The final MLWHF score is gathered by adding the scores for the questions.

New York Heart Association class was expressed on the four-grade scale in regard to whether the patient felt tiredness, breathlessness or heart palpitation and according to the level of strain at which the patient experienced the symptoms.

Six-Minute Walk Tests (6MWT) - This test required from the patients to cover the greatest possible distance during the 6-minute walk. It is normal for a healthy person to cover the distance of 400 to 700 m. After therapy, the patients were expected to increase this distance by 70-120 m or 12-40%.

Left ventricular ejection fraction was assessed by echocardiography, and the latter was done at the Department of Cardiology, Medical Center Zaječar. The equipment applied was VVID 3 GE Medical Systems. LVEF was calculated by Teicholtz formula:

$$EF = \frac{EDV\ (\text{end-diastolic volumen}) - ESV\ (\text{end-systolic volumen})}{EDV}$$

(a similar formula to the above mentioned, but without the background activity). Reference values of LVEF were 62±8%, with lower limit set at 54%.

All the functional capacity tests along with echocardiography were done in all the patients at the beginning of the follow-up period and after12 months.

All statistical analyses were performed in SPSS 12.0 (SPSS Inc, Chicago, Illinois) statistical package. The results were presented as frequency, percentage and mean±SD. The X^2, Mann-Whitney U test and T test were used to compare the two groups. T test and Wilcoxon Signed Ranks test were used to test differences of paired samples. All p values less than 0.05 were considered significant.

Results

According to the tests performed, there was no difference between the groups at the beginning of the study regarding their sex, age, BMI (body mass index), VVI to DDD pacemaker implantation ratio, or functional capacity. The only difference between groups was in the QRS duration in pacemaker stimulation. QRS was statistically significantly shorter in the RVOT group **(Table 1)**.

The average LVEF was within the normal range, and did not show any statistically significant difference between groups at the beginning of the study. After 12 months of pacemaker stimulation, LVEF remained the same regardless of lead position, 60.96±10.56% (p=0.31) in RVA group, and 57.77±10.86% (p=0.27) in RVOT group **(Table 2)**.

Table 1. Comparison of the patients from the RVA and RVOT group at the beginning of study. BMI (Body Mass Index), QRSs - QRS duration in intrinsic rhythm (sensing), QRSp - QRS duration in pacemaker stimulation
Tabela 1*. Komparacija grupe sa elektrodom u vrhu desne komore (RVA) i grupe sa elektrodom u izlaznom traktu desne komore (RVOT), na uključivanju u studiju. BMI (Indeks telesne mase), QRSs - širina QRS kompleksa za vreme intrizing ritma (sensing), QRSp – širina QRS kompleksa u stimulaciji pejsmejkerom, 6-MTH – šestominutni test hodanja*

Parameters *Parametri*	RVA group/*grupa* N=61	RVOT group/*grupa* N=71	Test and statistical significance *Test i statistička značajnost*
Men/*Muškarci*	43 (70.50%)	46 (64.78%)	p=0.48
Women/*Žene*	18 (29.50%)	25 (35.22%)	
Age/*Godine starosti*	72.72±9.40	72.69±8.66	p=0.98
BMI	26.47±4.48	27.09±4.33	p=0.42
QRSs (ms)	91.15±20.33	88.87±22.90	p=0.23
QRSp (ms)	151.34	126.34	p<0.001
VVIR	26 (42.62%)	35 (49.29%)	p=0.44
DDDR	35(57.38%)	36 (50.71%)	
MLWHF score/*Skor*	44.65±20.72	39.98±18.11	p=0.18
NYHA I	14 (22.58%)	16 (22.22%)	p=0.66
NYHA II	19 (30.64%)	24 (33.35%)	
NYHA III	17 (27.43%)	21 (29.16%)	
NYHA IV	11 (19.35%)	10 (15.27%)	
6-MWT (m)/*6-MTH*	439.30±214.79	484.13±208.45	p=0.29
EF/*EF* (%)	59.16±10.43	59.55±11.40	p=0.85

EF - ejekciona frakcija

MLWHF Score

After 12 months of pacemaker stimulation, both groups had the same rate of improvement in MLWHF score. In the RVA group, MLWHF score improved from 44.65±20.72 to 32.76±21.02 (p<0.0001). The patients from the RVOT group achieved smaller, but still statistically significant improvement, from 39.98±18.11 to 32.37±23.56 (p<0.006) **(Table 2)**.

NYHA Class

After 12 months of pacemaker stimulation, both groups made the same statistically significant improvement of NYHA class. The majority of patients from the RVA group had a statistically significant improvement, and thus fell into NYHA class I, meaning that 36 patients (67.92%) were in NYHA class I, and 9 (16.98%) in class II (p<0.001). The same tendency of statistically significant NYHA class improvement was also observed in the RVOT group, where 32 patients (54.23%) were in class I, and 19 (32.22%) were in class II (p<0.001) **(Table 2)**.

6 MWT

After 12 months of pacemaker stimulation, the patients from the RVA group walked 531.59±272.30 m or 20.95% (p=0.03) more in comparison to the

Table 2. Comparison of the patients from the RVA and RVOT group after 12 month pacemaker stimulation
Tabela 2*. Komparacija grupe sa elektrodom u vrhu desne komore (RVA) i grupe sa elektrodom u izlaznom traktu desne komore (RVOT), nakon 12 meseci stimulacije pejsmejkerom*

Parameters *Parametri*	RVA group/*grupa* N=61		Test and statistical significance *Test i statistička značajnost*	RVOT group/*grupa* N=71		Test and statistical significance *Test i statistička značajnost*
	1. month/*mesec*	12. month/*mesec*		1. month/*mesec*	12. months/*meseci*	
MLWHF score/*skor*	44.65±20.72	32.76±21.02	p<0.0001	39.98±18.11	32.37±23.56	p<0.006
NYHA I	14 (22.58%)	36 (67.92%)	p<0.001	16 (22.22%)	32 (54.23%)	p<0.001
NYHA II	19 (30.64%)	9 (16.98%)		24 (33.35%)	19 (32.22%)	
NYHA III	17 (27.43%)	7 (13.22%)		21 (29.16%)	5 (8.47%)	
NYHA IV	11 (19.35%)	1(1.88%)		10 (15.27%)	3 (5.08%)	
6-MWT (m)/*6-MTH*	439.30±214.79	531.59±272.30	p=0.03	484.13±208.45	550.04±254.73	p=0.09
EF/*EF* (%)	59.16±10.43	60.96±10.56	p=0.31	59.55±11.40	57.77±10.86	p=0.27

EF - ejekciona frakcija, 6-MTH - šestominutni test hodanja

starting values, whereas the patients from the RVOT group walked 550.04±254.73 m, but that was only 13.63% (p=0.09) longer distance than the starting values **(Table 2)**.

Discussion

Our research focused on comparing different pacemaker lead positions in the right ventricle, regarding its influence on the functional status in the patients who had the preserved left ventricular function at the beginning of the study and also after 12 months in real life circumstances.

The authors have concluded on the basis of the literature data published so far that the QRS duration in RVOT stimulation is shorter than in RVA stimulation [8–21]. Other authors also showed that QRS duration in the patients from the RVOT group was statistically significantly shorter than in the patients from the RVA group, but it had no protective effect on the left ventricular systolic function. LVEF was the same in the RVA group, and it even deteriorated slightly in the RVOT group after 12 months of pacemaker stimulation. Our results even showed a negative effect on diastolic function in the RVOT group compared to the RVA group [21]. Protect-Pace study also showed that lead position in the right ventricle had no protective effect on left ventricular function regardless of the QRS duration [22]. Although RVOT position yielded a shorter QRS, it has not led to an improvement in LVEF or the patient's functional capacity in any of the studies performed. The majority of performed studies analyzing the influence of pacemaker stimulation from RVA vs. RVOT on the functional status were done in the patients with decreased LVEF. ROVA study, with 103 patients with heart failure, found no difference in the life quality [9]. Some CRT studies found an improvement in functional capacity [23], while in others there was no difference in NYHA class between the patients with RVA and the patients with RVOT stimulation [24]. A review article analyzed data from three studies which had followed up 800 patients with ventricle pacemaker lead in RVA or RVOT, regardless of the LVEF for 3 years. This analysis confirmed the influence of pacemaker lead positioning in the right ventricle on the distance in 6 MWT [25]. Tse et al. performed a study involving patients with the preserved LVEF, which had their lead position changed from RVA to RVOT on pulse generator change, and they found that after 18 months of follow-up, the distance they were able to cover was significantly longer [18].

In contrast to the above mentioned studies, our results suggested 6 MWT to be an "objective" measure of functional status. By measuring the distance covered by the patient it showed a statistically significant improvement only in the RVA group who had walked a distance longer for 20.95% (p=0.03), whereas the RVOT group patients covered a distance longer only by 13.63% (p=0.09), which was not statistically significant. Our earlier results also showed an advantage of RVA stimulation over RVOT [26].

In our research we also found an equal improvement in "subjective" functional tests MLWHF score and NYHA class in both RVA and RVOT groups, after 12 months of pacemaker stimulation.

Conclusion

Analysis of tests of functional status, such as New York Heart Association class and Minnesota Living With Heart Failure questionnaire, showed an even improvement in the patients from the right ventricle apex and right ventricular outflow tract group. Analysis of 6 Minute Walk Test showed that only the patients with the preserved left ventricular ejection fraction from the right ventricle apex group had a significant improvement after 12 months of pacemaker stimulation.

References

1. Prinzen FW, Strik M, Regoli F, Auricchio A. Basic physiology and hemodynamics of cardiac pacing. In: Ellenbogen K A, editor. Clinical Cadiac Pacing, Defibrillation, and Resynhronization Therapy. 4th ed. Philadelphia: Elsevier Saunders; 2011. p. 203-233.

2. Sweeney MO, Prinzen W. Ventricular pump function an pacing -psysiological and clinical integration. Circ Arrhythmia Electrophysiol. 2008;1:127-39.

3. Lewicka-Nowak E, Dabrowska-Kugacka A, Tybura S, Krzymińska-Stasiuk E, Wilczek R, Staniewicz J, et al. Right ventricular apex versus right ventricular outflow tract pacing: prospective, randomised, long term clinical and echocardiographic evaluation. Kardiol Pol. 2006;10:1082-91.

4. Bharat V, Prakash B, Das NK. RVOT pacing versus roght ventricle apical pacing: Implantation Experience and ECG Characteristics. 2009:1-7. Available from: http://cme.mcgill.ca. http://university-cme.ca,http://www.university-cme.ca/canada/index.php?lang=fr.

5. Brignole M, Auricchio A, Baron-Esquivias G, Bordachar P, Boriani G, Breithardt OA, et al. 2013 ESC Guidelines on cardiac pacing and cardiac resynchronization therapy. The Task Force on cardiac pacing and resynchronization therapy of the European Society of Cardiology (ESC). Developed in collaboration with the European Heart Rhythm Association (EHRA). European Heart Journal. 2013;1-49.

6. Bacior B, Loster M, Klocek M. Quality of Life in Patients wuth Implantable Cardiac Devices. In: Kawecka-Jaszck K, editor. Healt Related Quality of Life in Cardiovascular Patients. Italia: Springer-Veriag; 2013. p. 91-103.

7. Cano OJ, Osca MJ, Sancho-Tello JM. Comparison of effectiveness of right ventricular septal pacing versus right ventricular apical pacing. The American Journal of Cardiology. 2010;10:1426-32.

8. Schwaab B, Frohling G, Alexander C, Kindermann M, Hellwig N, Schwerdt H, et al. Infulence of right ventricular stimulation site on left ventricular function in atrial syncronous ventricular pacing. J Am Coll Cardiol. 1999;33:317-23.

9. Stambler BS, Ellenbogen K, Zhang X, Porter TR, Xie F, Malik R, et al; ROVA Investigators. Right ventricular outflow versus apical pacing in pacemacer patients with congenstive

heart failure and atrial fibrillation. J Cardiovasc Electrophysiol. 2003;14:1180-6.

10. Tse HF, Yu C, Wong KK, Tsang V, Leung YL, Ho WY, et al. Functional abnormalities in patients with permanent right ventricular pacing: the effect of sites of electrical stimulation. J Am Coll Cardiol. 2002;40:1451-8.

11. Hillock RJ, Stevenson IH, Mond HG. The right ventricular outflow tract: a comparative study of septal, anterior wall, and free wall pacing. Pacing and Clinical Electrophysiology. 2009;32:172-6.

12. Padeletti L, Lieberman R, Schreuder J, Michelucci A, Collella A, Pieragnoli P, et al. Ventricular pacing lead location alters systemic hemodynamics and left ventricular reduced ejection fraction. J Am Coll Cardiol. 2006;48:1634-44.

13. Ten Cate TJ, Scheffer MG, Sutherland GR, Verzijlbergen JF, Van Hemel NM. Right ventricular outflow and apical pacing comparably worsen the echocardiographic normal left ventricle. Eur J Echocardiography. 2008;9:672-7.

14. Kristiansen HM, Vollan G, Hovstad T, Keilegavlen H, Faerestrand S. Right ventricular pacing and sensing function in high posterior septal and apical lead placement in cardiac resynhronization therapy. Indian Pacing and Electrophysiology Jurnal. 2012;12:4-14.

15. Kypta A, Steinwender C, Kammler J, Leisch F, Hofmann R. Long-term outcomes in patients with atrioventricular block undergoing septal ventricular lead implantation compared with standard apical pacing. European Heart Journal. 2008;10:574-9.

16. Weizong W, Zhongsu W, Yujiao Z. Effects of right ventricular nonapical pacing on cardiac function: a meta-analysis of randomized controlled trials. Pacing and Clinical Electrophysiology 2013; Article published online: 25 feb 2013.

17. Mazza A, Bendini MG, Leggio M. Incidence and predictors of heart failure hospitalization and death in permanent pacemaker patients: a single-centre experience over medium-term follow-up. Europace. First published online: Feb 26,2013.

18. Tse HF, Wong KK, Siu CW, Zhang XH, Ho WY, Lau CP. Upgrading pacemaker patients with right ventricular apical pacing to right ventricular septal pacing improves left ventricular performance and functional capacity. J Cardiovasc Electrophysiol. 2009;20:901-5.

19. Nakamura H, Mine T, Kanemori T, Ohyanagi M, Masuyama T. Effect of right ventricular pacing site on QRS width. Asian Cardiovasc Thorac Ann. 2011;19:339-45.

20. Wang F, Shi H, Sun Y, Wang J, Yan Q, Jin W, et al. Right ventricular outflow pacing induces less regional wall motion abnormalities in the left ventricle compared with apical pacing. Europace. 2012;14:351-7.

21. Mitov V, Perišić Z, Jović A, Adamović D, Zastranović L, Aleksić A, et al. The effect of right ventricle pacemaker lead position on diastolic function in patients with preserved left ventricle ejection fraction. Hell J Nucl Med. 2013;16(3):204-8.

22. Kaye GC, Linker NJ, Marwick TH, Pollock L, Graham L, Pouliot E, et al. Effect of right ventricular pacing lead site on left ventricular function in patients with high-grade atrioventricular block: results of the Protect-Pace study. European Heart Journal. First published online: 4 September 2014.

23. Morales MA, Startari U, Rossi G, Panchetti L, Rossi A, Piacenti M. Reverse left ventricular remodeling is more likely in non ischemic cardiomyopathy patients upgraded to biventricular stimulation after cronic right ventricular pacing. Cardiovascular Ultrasound. 2011;9:41-3.

24. Haghjoo M, Bonakdar HR, Jorat MV, Fazelifar AF, Alizadeh A, Ojaghi-Haghjghi Z et al. Effect of right ventricular lead location on responce to cardiac resynchronization therapy in patients with end-stage heart failure. Europace. 2009;11:356-63.

25. Kaye G, Stambler BS, Lee R. Search for the optimal right ventricular pacing site: desing and implementation of three randomized multicenter clinical trials. Pacing Clin Electrophysiol. 2009; 4:426-33.

26. Mitov V, Perišić Z, Jović A, Adamović D, Zastranović L, Aleksić A, et al. The efect of pacemaker lead positioning on left ventricle function and patient functional capacity. Eur J Heart Fail. 2012;11:389.

Rad je primljen 2. XII 2014.
Recenziran 2. II 2015.
Prihvaćen za štampu 15. VI 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:212-216.

General Hospital Subotica, Department of Orthopedics and Traumatology[1]
Health Center Krupanj, Krupanj[2]
Institute for Child and Youth Health Care, Novi Sad
Department of Orthopedics and Traumatology[3]
Clinical Centre of Vojvodina, Novi Sad
Department of Orthopedics and Traumatology[4]

Original study
Originalni naučni rad
UDK 616.728.3-001-08
DOI: 10.2298/MPNS1608217R

CONCOMITANT INJURIES OF ANTERIOR CRUCIATE LIGAMENT AND MENISCUS

UDRUŽENOST POVREDA PREDNJEG UKRŠTENOG LIGAMENTA I POVREDA MENISKUSA

Vladimir RISTIĆ[1], Mirsad MALJANOVIĆ[1], Ivan MIHAJLOV[2], Vukadin MILANKOV[3] and Vladimir HARHAJI[4]

Summary

Introduction. The aim of this study was to determine the correlation between meniscal injuries with injuries of the anterior cruciate ligament, as well as risk factors for those associated injuries. **Material and Methods.** This study included 496 operated patients. Almost half of patients with meniscal injury were between the ages of 21 and 30 years. **Results.** Meniscal injuries were diagnosed in 187 patients (38%). These patients were significantly older than the patients without meniscal injury. Meniscal injuries were significantly more frequent in patients who played sports recreationally than in professional athletes. The patients with meniscal injury underwent surgery almost four months later than the patients with preserved menisci. Meniscal injuries occurred significantly more frequently by non – contact mechanism, as a result of landing and sudden changes of direction and rhythm of running. **Conclusion.** Male patients hurt the medial meniscus more often, "bucket handle" type of lesion being much more frequent than on the lateral meniscus. The increase of body mass index is exactly proportional to the increase in the incidence of meniscal injuries.

Key words: Anterior Cruciate Ligament; Knee Injuries; Anterior Cruciate Ligament Reconstruction; Menisci, Tibial; Risk Factors; Age Factors; Reacreation; Sports; Body Mass Index

Sažetak

Uvod. Cilj istraživanja bio je da utvrdimo povezanost povreda prednjeg ukrštenog ligamenta i povreda meniskusa, kao i faktore rizika za nastanak ovih udruženih povreda. **Materijal i metode.** Ispitivanu grupu činilo je 496 operisanih pacijenata sa povredom prednjeg ukrštenog ligamenta. Gotovo polovina pacijenata sa povredom meniskusa pripadala je starosnoj grupi između 21 i 30 godina. **Rezultati.** Povrede meniskusa su dijagnostikovane kod 187 pacijenata (38%). Pacijenti sa povredom meniskusa bili su statistički značajno stariji od pacijenata sa intaktnim meniskusima. Povrede meniskusa su se statistički signifikantno češće dešavale kod pacijenata koji se sportom bave rekreativno, nego kod profesionalnih sportista. Pacijenti sa povredom meniskusa su operisani gotovo četiri meseca kasnije u odnosu na pacijente sa očuvanim meniskusima. **Zaključak.** Udružene povrede prednjeg ukrštenog ligamenta i meniskusa najčešće nastaju nekontaktnim mehanizmom. Muškarci češće povređuju unutrašnji meniskus po tipu *bucket handle* nego na spoljašnjem meniskusu. Porast indeksa telesne mase upravo je srazmeran porastu incidencije udruženih povreda meniskusa.

Ključne reči: prednji ukršteni ligament; povrede kolena; rekonstrukcija prednjeg ukrštenog ligamenta; tibijalni meniskusi; faktori rizika; faktori starosti; rekreacija; sport; indeks telesne mase

Introduction

Anterior crucate ligament (ACL) injuries are commonly encountered in athlete population. According to the available epidemiological data in the United States, the annual incidence of ACL injuries is estimated to range from 100,000 to 250,000 injuries per year, and more than 70% of these injuries are suffered by the sports active population [1, 2]. In Vojvodina, which has about two million inhabitants, about 400 reconstructions of the ACL (2 reconstructions per 10.000 residents) are performed per year. As for the European countries, the most accurate data are those from Denmark, where during one year three injuries per 10,000 residents are recorded, with a higher frequency of occurrence among the athletes as well [3]. These data are very similar to the data from the United States, where it is estimated that injuries occur in one out of 3,000 people in the general population [1]. The reason for this epidemiological situation certainly lies in the fact that the number of participants in sports on the global level is constantly growing. Not only have sports activities become an important part of modern life, but also an increasing number of people go in for sports for relaxation and entertainment. The number of men and women who become members of fitness and various sports clubs is increasing [4]. All this supports the fact that a significant increase in injuries of ACL has been recorded in the past 10–15 years [5].

It is estimated that almost every second or third ACL injury goes with additional menuscus injury

Corresponding Author: Dr Vladimir Ristić, Opšta bolnica, Odeljenje za ortopediju i traumatologiju, 24000 Subotica, Izvorska 3, E-mail: ristic@tippnet.rs

Abbreviations
ACL – anterior cruciate ligament
BMI – body mass index

[6–8]. In acute cases lateral meniscus is most frequently injured, while chronic lesions are connected most often with medial meniscus [6, 7]. Both menisci are the second most important structures of stability of a knee joint. ALC is the first one. Levy and al. [8] have proved that bilateral total meniscectomy does not influence the knee stability significantly if ACL is uninjured. If it is ruptured, most of ACL function is taken over by medial meniscus in order to limit the anterior tibial translation, thus providing stability and retaining the biomechanics of knee joint.

The aim of this study was to determine the correlation between meniscal and ACI injuries, as well as risk factors for meniscal injury in patients with ACL rupture.

Material and Methods

With the permission of the Ethics Committee of the Clinical Center of Vojvodina, a prospective study was conducted at the Department of Orthopedic Surgery and Traumatology. The study included 496 patients with ACL ruptures operated between January 2012 and December 2013. Data were gathered from the history of injury, operative protocols and register of ACL injuries kept at the Department.

Of the total number of operated patients, 401 (80.8%) were male, while 95 (19.2%) were female. The average age was 25.54 ± 7.69 years (minimum 13, maximum 57). The criterion for inclusion in the study was the diagnosed ACL rupture in the patients who had got injured during active participation in sports and recreation, in the performance of daily activities or in traffic accidents.

The results are presented in tables and graphs. Several independent variables were observed and subgroups were formed accordingly as a part of the study sample. The comparison was carried out between certain subgroups of patients by Student's T-test, with marked statistical significance level of $p<0.05$.

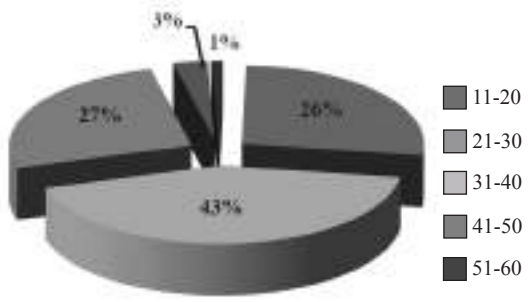
Results

Out of 496 patients with ACL rupture, 309 (62.3%) had no rupture of menisci, while the remaining 187 (37.7%) were diagnosed with meniscal lesions.

The medial meniscal lesion was found in 101 cases (20.4%), and 56 patients (11.3%) had lateral meniscal rupture. Both menisci were injured in 30 patients (6%).

The average age of patients with intact menisci was 24.7 years, while the average age of the patients with a damaged meniscus or menisci was 27 years. A statistical significance ($p <0.05$) was found by comparing these two values.

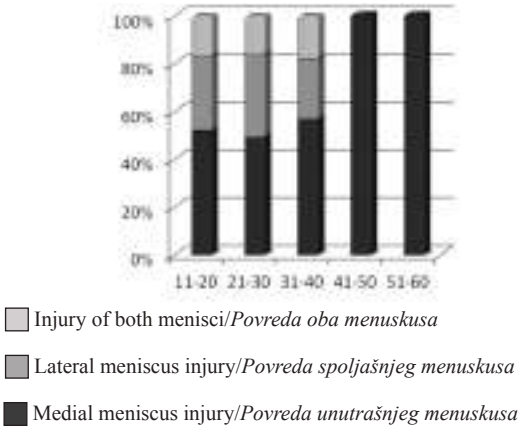
The patients were divided into five groups according to their age. Most patients with concomitant meniscus and ACL injury were in the age group from 21 to 30 years (43.3%) (**Graph 1**).



Graph 1. Age structure of the subjects with associated injuries
Grafikon 1. *Starosna struktura ispitanika sa udruženim povredama*

Comparing these associated injuries among different age groups, it was found that the patients aged between 31 and 40 years had associated medial meniscal injury significantly more often than the patients aged between 11 and 30 years ($p<0.05$). A statistical significance was also found considering lateral meniscal lesions, which happened more frequently in the patients under 40 years of age ($p<0.05$). Comparing the existence of associated ACL and medial or lateral meniscal ruptures within the age groups, the patients between 31 and 40 years and 41 and 50 years of age were found to be statistically significantly more likely to have ACL and medial meniscal injury ($p<0.05$) (**Graph 2**).

Out of all subjects, 480 (96.8%) were active in sports, while 16 (3.2%) did not go in for sports ($p<0.05$). In relation to sports activity, more subjects were engaged in recreational sports, i.e. 282 of them (58.75%), while 198 were professional athletes (41.25%). As for the patients with a meniscal lesion, 117 (62.6%) were engaged in recreational sports, 63 (33.7%) practiced



Graph 2. Meniscal injuries in relation to age structure
Grafikon 2. *Povrede meniskusa u odnosu na starosnu strukturu*

Table 1. Meniscal lesions depending on sport
Tabela 1. *Lezije meniskusa u zavisnosti od vrste sporta*

	Medial meniscus *Unutrašnji meniskus*	Lateral meniscus *Spoljašnji meniskus*	Both menisci *Oba meniskusa*	Both menisci intact *Intaktna oba*
Basketball/*Košarka*	15.5%	15.5%	8.3%	60.7%
Soccer/*Fudbal*	19.6%	10.4%	5%	65%
Volleyball/*Odbojka*	33.3%	11.1%	5.6%	50%
Handball/*Rukomet*	12.8%	10.6%	6.4%	70.2%
Martial arts/*Borilačke veštine*	31.3%	12.5%	9.3%	46.9%
Skiing/*Skijanje*	4%	4%	4%	88%
Am. Football/*Ragbi*	25%	25%	0%	50%
Other/*Drugi sport*	45%	5%	0%	50%
Non-athletes/*Nesportisti*	27.3%	13.6%	13.6%	45.5%

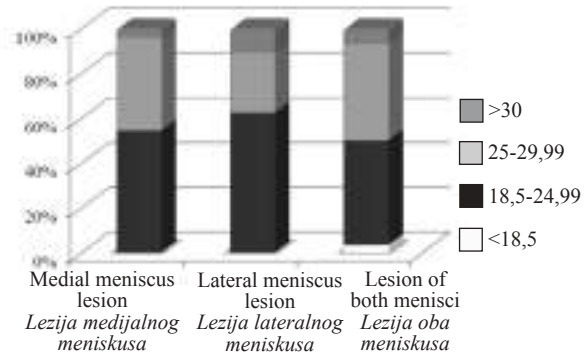
sports professionally, while 7 3.7%) were not active in any sport. A statistically significant difference ($p<0.05$) was found by comparing the level of sports between the group of patients with meniscal injury and the group of patients with intact menisci.

Injuries of medial meniscus are often present among patients who are active in „other" sports, such as: water polo, athletics, gymnastics, tennis. Lateral meniscal rupture occurred most often in the patients who were active in rugby or American football, while both meniscal injuries were found in the group of patients who were not engaged in any kind of sports activity. The highest percentage of intact menisci happened in those patients who had isolated ACL injury during skiing (**Table 1**).

Nutritional status of our patients was determined according to their anthropometric data – body height and weight. The patients were divided into four groups: normal weight ($18.5 \leq$ body mass index (BMI) <25), malnourished (BMI <18.5 kg/m^2), overweight ($25 \leq$ BMI < 30) and obese (BMI>30 kg/m^2). Most of them had normal weight (62.1%), 32.3% of the patients were overweight, 4.6% of them were obese, whereas only 1% of the patients were malnourished.

A medial meniscal injury associated with ACL rupture was found more frequently in overweight patients than in those with normal weight ($p<0.05$). A statistically significant difference between these two groups was observed ($p<0.05$) when comparing the total number of medial and lateral meniscal injuries, and it was in favor of those patients who had a body mass index greater than 25 (**Graph 3**).

Three groups of patients were formed according to the time elapsed from the moment of ACL rupture to surgical intervention. The majority of patients (304, i.e. 61.2%) were operated within six months after injury. The ACL reconstruction was performed between 6 and 12 months after rupture in 144 cases (29.1%), while surgery was performed in 48 patients (9.7%) more than a year after the injury. The longer the period between getting injured and being operated, the higher was the number of associated meniscal injuries. As for the average time elapsed from getting injured and surgical intervention, there was

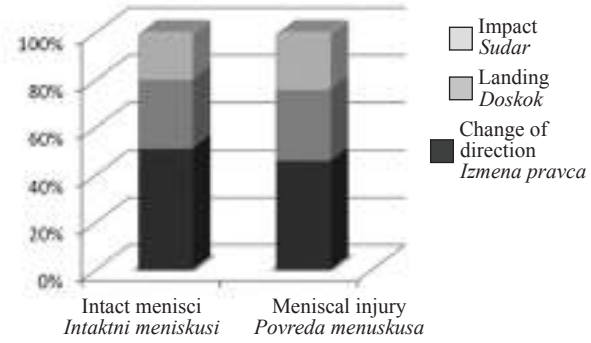


Graph 3. Meniscal lesions depending on BMI
Grafikon 3. *Lezije meniskusa u odnosu na indeks telesne mase*

also a statistically significant difference between the groups of patients with an injury of meniscus (on average after 10.75 months of delayed surgery) and those patients with an intact menisci (operated on average 7 months after injury).

A sudden change of direction was the most common cause of injury. It happened in 244 subjects (49.2%); 145 patients (29.2%) were injured during landing, while in 107 patients (21.6%) injury occurred by contact mechanism and collision. There was a statistically significant difference ($p<0.05$) when comparing the mechanisms of ACL injury between the group of patients with intact menisci and those diagnosed with meniscal lesion (**Graph 4**). The associated ACL and meniscal injury occurred significantly more often during non-contact injuries (changes in direction or landing) than in contact way (crash of opponent players) (75.8%: 24.2%).

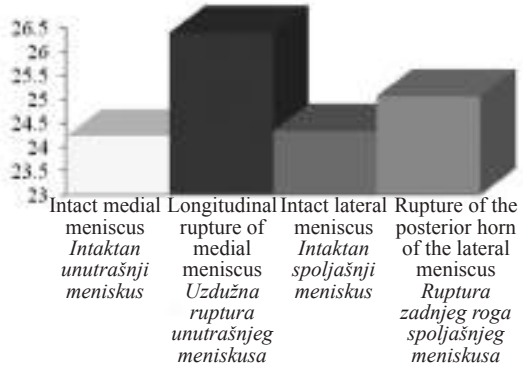
The comparison of subjects regarding the localization of meniscal lesions revealed a statistical significance in relation with the body mass index between the patients with longitudinal medial meniscal rupture and those with intact medial meniscus ($p<0.05$). Being overweight significantly increases the chances of associated injuries of ACL and posterior horn of lateral meniscus ($p<0.05$) (**Graph 5**). On the other



Graph 4. Meniscal lesions in relation to mechanism of ACL rupture
Grafikon 4. *Lezije meniskusa u odnosu na mehanizam rupture prednjeg ukrštenog ligamenta*

hand, the age of the participants had a statistically significant influence on the injury of the posterior horn of the medial meniscus, while not significantly affecting the localization of lateral meniscus lesions.

As for the concomitant ACL and meniscus injuries between men and women, there was no statistically significant difference ($p>0.05$). However, male patients injured their medial meniscus more frequently than the lateral meniscus ($p<0.05$). In male patients with associated injuries of ACL and meniscus, "bucket handle" meniscal lesion occurred also more often on the medial meniscus than on the lateral one ($p<0.05$), while no significant difference was observed in women in this respect.



Graph 5. Average BMI among types of meniscal lesions
Grafikon 5. *Prosečan indeks telesne mase među tipovima lezije meniskusa*

Discussion

It has been thought for a long time that ACL reconstructions prevent degenerative changes in articular cartilage, which has not been confirmed by later studies [9]. However, they significantly affect the stability of the knee joint, reduce subsequent meniscal injuries, provide return to sports activities and improve the quality of life of patients [6, 9–11].

More than one-third of our patients (37.7%) with ruptured ACL had associated lesion of the meniscus. This finding is consistent with the literature [12–17], stating that a meniscus injury occurs in 35–79% of patients with ACL rupture.

Associated injuries are more common in men. In this study, 80% of patients were male. In other similar studies [19, 20] men are also more affected, the percentage ranging from 70 to 80%. A significantly higher proportion of the male population having this problem is attributed to the greater involvement of men in professional sports, especially in contact sports, where the forces acting on the knee joint are extremely strong. The results of this study show that there is no significant correlation between meniscal injuries and the sex of patients, in contrast to the results of a study conducted by the Kluczynski et al. [21], where it was found that male gender was correlated with significantly more frequent injuries, of both medial and lateral meniscus [16, 17, 19, 20]. Medial meniscal ruptures also dominated among our male patients.

The average age of the surveyed patients was 25.5 years (ranging between 13 and 57 years). The average age of patients with intact menisci was 24.7 years, while the patients with meniscus and ACL injury were 27 years old on average. The largest number of patients with meniscus lesion, almost half of the total, belongs to the population between 21 and 30 years of age [4, 14–17, 22].

Adequate preparation of all muscle groups and joints before any sports activity is a significant measure of injury prevention, especially among amateurs, who do not pay enough attention to this issue. Although Myklebust et al., as well Bjordal et al. [9, 23] found associated meniscus and ACL injuries more frequently in professional athletes; however, these injuries are more common in recreational athletes in our study sample. A possible reason for the frequent occurrence of meniscus lesions during professional career can be explained by competitive character and large number of rough starts, and strong wish to achieve better sports results [24]. On the other hand, our result is logical considering the fact that professional athletes are well prepared in full training and physically ready for extreme efforts. Besides, professional athletes are under constant control of club doctors and medical staff, so an ACL rupture is diagnosed early, it is monitored actively and treated to full recovery, thus reducing the occurrence of later complications, above all, meniscal injury.

Associated injuries occur more frequently by non-contact injury mechanism, such as landing or changes of direction, as well as by direct contact with other players. A multidisciplinary approach to non-contact knee injuries mostly speaks in favor of movements of the whole body that participate in the mechanism of injury [14, 25, 26]. The combination of anterior translation of tibia in relation to the fe-

mur and dynamic valgus position predict the injury, which may also include other joints of the lower extremities [25, 27].

The lateral meniscus usually gets injured in patients who have practiced contact sports such as rugby and American football. This result indicates an increase in the incidence of meniscal injuries in general, especially during practicing these sports, which is similar to the results of a study conducted by Chomiak et al. [28]. Medial meniscal tears occurred most often in our patients involved in "other sports" such as tennis, water polo, swimming, athletics and gymnastics. Besides water polo, other sports are non-contact, so we can conclude that the injuries of medial meniscus are largely associated with contactless mechanism of injury. These data correlate with data from the literature [14, 29]. Patients who are not engaged in any kind of sport activity are less exposed to injuries, such as ACL and meniscus [14, 29]. However, the simultaneous ruptures of both menisci and ACL are most frequent in this group of patients. The reason for such a result can be found in the fact that they are physically unfit and each provocative position of their knee joint can lead to lesions of some of its structures. However, a delayed diagnosis of ACL rupture is more frequent in those who do not go in for any sports than among athletes [14, 29]. ACL injury without meniscal lesions appeared most often in the patients who were injured while skiing, which globally carries a high risk for injury, although it is significantly more present in the Scandinavian countries and in United States [5, 9, 23, 30].

The dimensions of the body, such as height, weight, body fat content and body mass index were evaluated in several studies [31–34], suggesting that higher levels of body mass index may be a potential risk factor for non-contact injuries. Hypothetically, the higher value of body mass index may increase the force that is transmitted to the ligament apparatus of the knee joint and stabilizer muscles [35, 36]. Our results suggest that patients with a body mass index greater than 25 kg/m^2 have an associated medial meniscal tear significantly more often. Associated ACL injuries with ruptures of medial meniscus or tears of posterior horn of lateral meniscus are directly connected to obesity.

The injured knee joint requires timely and accurate diagnosis, which is a prerequisite for surgical intervention on time. Hagino et al. [6] registered the incidence of associated ACL and meniscus injuries in as many as 79.2% of cases, with a significant difference compared to our study because their patients had significantly more injuries of the lateral meniscus. In acute trauma, the incidence was lower (72.7% vs. 84.8% for the chronic ACL rupture). Regarding the locations of meniscal tears in acute trauma, the medial meniscus was injured only in 10.8% of cases, the lateral in 69.4% and 19.9% of patients had the rupture of both menisci. The situation was different in case of chronic injuries. In our study sample, 24.7% of patients had medial meniscus tears, 33.9% of them had lateral meniscus tears and 41.4% of patients had both ruptures [6]. The similarity between the results of our study and the above mentioned study is only in the conclusion that the lateral meniscal tear was commonly associated with an acute ACL injury, while medial meniscal tear with the chronic one.

Delay in ACL reconstruction is associated with more severe and painful meniscal and chondral injuries [18]. However, there are opposite opinions. Michalitsis et al. [13] believe that if ACL reconstruction is performed more than a year after the injury, there is a greater chance for serious cartilage damage, but without increasing the incidence of meniscal injuries. In our study, the average length of time elapsed between injury and surgical intervention in the patients with additional meniscal lesion was nearly 11 months, while in the patients with intact menisci it was significantly shorter (7 months). The results show that the percentage of intact meniscus decreases with time, while medial meniscus lesions and injuries of both menisci significantly increase. After ACL tearing, which is the most important stabilizer of the knee, other structures of the joint are overloaded by redistributing the forces, so there is a direct physical contact between the femoral condyle and meniscus. This results in degenerative damage of menisci and articular cartilage in the first place [13, 18, 37]. Lateral meniscal tear occurs more acutely, in a short period of time after ACL injury, while medial meniscus is most often injured if the period of time between the injury and surgical reconstruction of the ligament is longer [6, 36, 38–40]. Among the patients who underwent surgery within 6 months after injury, the percentage of medial meniscus rupture increases over the time while the percentage of lateral one stagnates, even decreases slightly. The reasons for these results could be due to the localization and anatomical characteristics of the menisci, as well as the relationship of medial and lateral meniscus with the anterior cruciate ligament, or different mechanisms of meniscus lesions [38].

Cerabona and Keene [41, 42] believe that the longitudinal rupture of medial meniscus is the most frequent injury associated with ACL injury. In our study sample, the most frequent location happens to be posterior horn tears of medial or lateral menisci. The patients with posterior horn tears of medial meniscus were on average four years older than the patients with intact meniscus. Among male patients, the longitudinal medial meniscal rupture occurs much more frequently than the rupture of lateral meniscus. In addition, incarcerated "bucket handle" type of rupture, which is a variant of the longitudinal rupture, occurs more frequently in medial meniscus in male patients. This result can be explained by different mechanisms of injuries, as well as by playing different sports at different sports surfaces [14, 25, 30].

One of the main drawbacks of this research is the intraoperative diagnosing of meniscal tears as well as the specific type and localization of rupture. Unfor-

tunately, although magnetic resonance imaging (MRI) can diagnose the localization of meniscal lesion with high certainty, this method is not available to all patients due to its high cost. The waiting lists in our public institutions are long and ligament apparatus injuries of the knee are not on the priority list of institutions. However, the value of this research is in its suggesting directions for future research, which could determine the temporal relationship with a specific type of meniscal lesions.

Conclusion

The meniscal injuries are associated with anterior cruciate ligament injuries to a very significant extent. They occur most often in sports activities, with no contact with opposing players. Concomitant injuries of anterior cruciate ligament and medial meniscus (posterior horn) frequently occur in obese men from the age group from 30 to 40, who are engaged in sports and in those who had their ligament reconstructed with a delay.

References

1. Silvers HJ, Mandelbaum BR. Prevention of anterior cruciate ligament injury in the female athlete. Br J Sports Med 2007;41:52-9.
2. Bradley JP, Klimkiewicz JJ, Rytel MJ, Powell JW. Anterior cruciate injuries in the national football leauge: epidemiology and current treatment trend among physicians. Arthroscopy. 2002;18:502-9.
3. Kvist J. Rehabilitation following anterior cruciate ligament injury. Sports Med. 2004;34:269-82.
4. Majewski M, Nabelt S, Steinbruck K. Epidemiology of athletic knee injuries: a 10-year study. Knee. 2006;13:184-8.
5. Olsen OE, Myklebust L, Engerbretsen I, Holme R, Bahr R. Relationship between floor type and risk of ACL injury in team handball. Scand J Med Sci Sports. 2003;13:299-304.
6. Hagino T, Ochiai S, Senga S, Yamashita T, Wako M, Ando T, et al. Meniscal tears associated with anterior cruciate ligament injury. Arch Orthop Trauma Surg 2015;135:1701–6.
7. Wyatt RWB, Inacio MCS, Liddle KD, Maletis GB. Factors associated with meniscus repair in patients undergoing anterior cruciate ligament reconstruction. Am J Sports Med. 2013;20:41-6.
8. Levy IM, Torzilli PA, Warren RF. The effect of medial meniscectomy on the anterior – posterior motion of the knee. J Bone Joint Surg Am. 1982;64:883-8.
9. Myklebust G, Engebretsen L, Braekken IH. Prevention of anterior cruciate ligament injuries in female team handball players: a prospective intervention study over three seasons. Clin J Sport Med 2003;13:71-8.
10. Ristić V, Ristić S, Maljanović M, Đan V, Milankov V, Harhaji V. Risk factors for bilateral anterior cruciate ligament injuries. Med Pregl 2015;68(5-6):192-7.
11. Ristić V, Ristić S, Maljanović M, Milankov V, Harhaji V, Đuričin A. Quality of life after bilateral anterior cruciate ligament reconstructions. Med Pregl 2015;68(9-10);308-15.
12. Rotterud JH, Sivertsen EA, Forssblad M, Engebretsen L, Aroen A. Effect of meniscal and focal cartilage lesions on patient: reported outcome after anterior cruciate ligament reconstruction. Am J Sports Med. 2013;41:535-43.
13. Michalitsis S. Vlychou M, Malizos KN, Thriskos P, Hantes ME. Meniscal and articular cartilage lesions in the acl – deficient knee: correlation between time from injury and knee scores. Knee Surg Sports Traumatol Arthrosc. 2013;1:1-8.
14. Ristić V, Ninković S, Harhaji V, Milankov M. Causes of anterior cruciate ligament injuries. Med Pregl 2010;63:541-5.
15. Milankov M, Miličić A, Savić D, Stanković M, Ninković S, Matijević R, et al. Revision anterior cruciate ligament reconstruction due to knee instability. Med Pregl. 2007;60(11-12):587-92.
16. Ristić V, Ninković S, Harhaji V, Stanković M, Savić D, Milankov M. Reconstruction of ACL by using two different techniques. Med Pregl. 2010;63(11-12):845-50.
17. Ninković S, Miličić A, Savić D, Stanković M, Radić S, Milankov M. Comparison of clinical and radiographic results of anterior cruciate ligament reconstructions. Med Pregl. 2006;59:421-5.
18. Fok AWM, Yau WP. Delay in ACL reconstruction is associated with more severe and painful meniscal and chondral injuries. Knee Surg Sports Traumatol Arthrosc. 2013;21:928-33.
19. La Prade R, Spiridonov S. Fibular Collateral Ligament Anatomical Reconstructions. Am J Sports Med. 2010;38:10.
20. Kyong HY, Dae KB. Anatomic reconstructive surgery for posterolateral instability of the knee. J Arthrosc Related Surg. 2006:22.
21. Kluczynski MA, Marzo JM, Bisson LJ. Factors associated with meniscal tears and chondral lesions in patients undergoing anterior cruciate ligament reconstruction. Am J Sports Med. 2013;20:11-7.
22. Chen G, Tang X, Li Q, Zheng G, Yang T, Li J. The evaluation of patient: specific factors associated with meniscal and chondral injuries accompanying ACL rupture in young adult patients. Knee Surg Sports Traumatol Arthrosc. 2013;10: 2227-33.
23. Bjordal JM, Arnly F, Hannestad B, Strand T. Epidemiology of anterior cruciate ligament injuries in soccer. Am J Sports Med. 1997;25:341-5.
24. Kobayashi H, Kanamura T, Koshida S, Miyashita K, Okado T, Shimizu T, et al. Mechanisms of the anterior cruciate ligament injury in sports activities: a twenty – year clinical research of 1,700 athletes. J Sports Sci Med. 2010;9:669-75.
25. Boden BP, Dean GS, Feagin JA, Garrett We Jr. Mechanisms of anterior cruciate ligament injuries. Orthopedics. 2000;23:573-8.
26. Frobell RB, Lohmander LS, Roos HP. Acute rotational trauma to the knee: poor agreement between clinical assessment and magnetic resonance imaging findings. Scan J Med Sci Sports. 2007;17:109-48.
27. Renstrom P. Non-contact ACL injuries in female athletes: an International Olympic Committee current concepts statement. Br J Sports Med. 2008;42:394-412.
28. Chomiak J, Junge A, Peterson L, Dvorak J. Severe injuries in football players. Influencing factors. Am J Sports Med. 2000;28:58-68.
29. Razi M, Salehi S, Dadgostar H, Cherati AS, Moghaddam AB, Tabatabaiand SM, et al. Timing of anterior cruciate ligament reconstruction and incidence of meniscal and chondral injury within the knee. Int J Prev Med. 2013;4:98-103.

30. Beynnon BD, Ettlinger CF, Johnson RJ. Epidemiology and mechanisms of ACL injury in alpine skiing. Understanding and Preventing Noncontact ACL Injuries. Am Orthop Soc Sports Med, Human Kinetics; 2007:183-8.

31. Arnason A, Sigurdsson SB, Gudmundsson A, Holme I, Engebretsen L, Bahr R. Risk factors for injuries in football. Am J Sports Med. 2004;32:5-16.

32. Ostenberg A, Roos H. Injury risk factors in female European football: a prospective study of 123 players during one season. Scand J Med Sci Sports. 2000;10:279-85.

33. Dvorak J, Junge A, Chomiak J, Baumann TG, Peterson L, Rosch D, et al. Risk factors analysis for injuries in football players. Possibillities for a prevention program. Am J Sports Med. 2000;28:69-74.

34. Faude O, Junge A, Kindermann W, Dvorak J. Risk factors in elite female soccer players. Br J Sports Med. 2006;40:785-90.

35. Murphy DF, Connolly DA, Beynnon BD. Risk factors for lower extremity injury: a review of the literature. Br J Sports Med. 2003;37:13-29.

36. Hwang BY, Kim SJ, Lee SW, Lee HE, Lee CK, Hunter DJ, et al. Risk factors for medial meniscus posterior root tear. The Am J Sports Med. 2012;40:1606-10.

Rad je primljen 22. II 2016.
Recenziran 7. III 2016.
Prihvaćen za štampu 28. III 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:217-223.

37. Greis PE, Bardana DD, Holmstrom MC, Burks RT. Meniscal injury: Basic science and evaluation. J Am Acad Orthop Surg. 2002;10:168-76.

38. Tandogan RN, Taser O, Kayaalp A, Taskiran E, Pinar H, Alparslan B, et al. Analysis of meniscal and chondral lesions accompanying anterior cruciate ligament tears: relationship with age, time from injury, and level of sport. Knee Surg Sports Traumatol Arthrosc. 2004;12:262-70.

39. Nikolić DK. Lateral meniscal tears and their evolution in acute injuries of the anterior cruciate ligament of the knee. Arthroscopic analysis. Knee Surg Sports Traumatol Arthrosc. 1998;6:26-30.

40. Snoeker BAM, Bakker EWP, Kegel CAT, Lucas C. Risk factors for meniscal tears: a systematic review including meta – analysis. J Orthop Sports Phys Ther. 2013;43:352-7.

41. Cerabona F, Sherman MF, Bonamo JR, Sklar J. Patterns of meniscal injury with acute anterior cruciate ligament tears. Am J Sports Med. 1988;16:603-9.

42. Keene G, Bickerstaff D, Paterson R. The natural history of meniscal tears in anterior cruciate insufficiency. Am J Sports Med. 1993;21:672-9.

University of Novi Sad, Novi Sad
Faculty of Medicine[1]
Clinical Center of Vojvodina, Novi Sad
Department of Psychiatry[2]

Original study
Originalni naučni rad
UDK 340.6:343.61(497.113)"1996/2005"
DOI: 10.2298/MPNS1608224G

SOCIODEMOGRAPHIC AND PSYCHIATRIC CHARACTERISTICS AMONG HOMICIDE OFFENDERS IN SERBIA - THE PROVINCE OF VOJVODINA (1996-2005)

SOCIODEMOGRAFSKE I PSIHIJATRIJSKE KARAKTERISTIKE POČINILACA UBISTVA U SRBIJI – POKRAJINI VOJVODINI (1996−2005)

Zoran GAJIĆ[1,2], Jovan MILATOVIĆ[2], Boris GOLUBOVIĆ[1,2], Josip DADASOVIĆ[1,2], Slađana RALEVIĆ[2] and Jagoš GOLUBOVIĆ[1]

Summary

Introduction. Recent studies have shown a growing correlation between violence and mental illness, but there is a higher risk of violent crimes only in certain cases of mental disorders. This study presents sociodemographic and psychiatric characteristics of homicide offenders in Serbia, in the Province of Vojvodina in a 10-year period (1996-2005). **Material and Methods.** The obtained data are based on performed forensic and psychiatric expert investigations of 154 homicide offenders in preceding period, considering sociodemographic data, personal history and current psychiatric status. Data were analyzed using the statistical John's Macintosh programe. **Results.** The male offenders were in the great majority (92%) as well as a low level of education (87%). A positive history of criminal acts was found in 24% of the subjects. Minority of subjects (21%) consumed alcohol on a daily basis. At the time of committing the crimes, 57% of homicide offenders were under the influence of alcohol, and just 2% of other psychoactive substances. Among the offenders who had previously received psychiatric treatment (31.2%), the most frequent diagnosis was alcohol addiction (25%) and anxiety disorders (22.9%). During the psychiatric examination 70.8% of the subjects were diagnosed with mental disorder: personality disorders (41%), alcohol addiction (84%), neurotic disorders (65%), schizophrenic psychosis (5.2%), affective disorders (3.2%), paranoid psychosis (2.6%), organic disorders (19%), psychoactive drug addiction (13%) and mental retardation (0.6%). Emotionally unstable personality disorder was dominant among personality disorders (55.6%). Diminished mental competency was established in 77.9% of subjects at the time of the homicide, being rather sever in most of them. All those diagnosed to have a psychotic disorder were mentally incompetent. **Conclusion.** Emotionally unstable disorders were the most common among the offenders who underwent forensic evaluation. A relatively low presence of psychotic disorders imposes the need for de-stigmatization particularly of the patients suffering from major mental illnesses.

Key words: Homicide; Violence; Forensic Psychiatry; Mental Disorders; Personality Disorders; Psychotic Disorders; Socioeconomic Factors; Crime; Alcoholism; Mental Competency

Sažetak

Uvod. Skorašnja istraživanja pokazuju sve veću povezanost između nasilja i duševnih oboljenja, ali je samo u određenim slučajevima mentalnih poremećaja prisutan povećan rizik za nasilne zločine. Ova studija predstavlja sociodemografske i psihijatrijske karakteristike počinilaca ubistva u Srbiji, Pokrajini Vojvodini, tokom desetogodišnjeg perioda (1996−2005). **Materijal i metode.** Podaci su dobijeni na osnovu sudsko-psihijatrijskih veštačenja 154 izvršioca ubistva u navedenom periodu, uzimajući u obzir sociodemografske karakteristike, prethodnu psihijatrijsku istoriju i trenutni psihijatrijski status. Za analizu podataka korišćen je statistički *John's Macintosh programe*. **Rezultati.** Među počiniocima dominiraju muškarci (92,2%) i osobe niskog obrazovnog nivoa (87%). Nađeno je da je 24% ispitanika već vršilo krivična dela. Alkohol je svakodnevno konzumiralo 21,4% ispitanih. Tokom vršenja zločina ubistva, 57,1% počinilaca bilo je pod dejstvom alkoholola, a samo 2,6% pod dejstvom drugih psihoaktivnih supstancija. Među onima koji su već bili pod psihijatrijskim tretmanom (31,2%), najčešće dijagnoze su bile zavisnost od alkohola (25%) i anksiozni poremećaji (22,9%). Psihijatrijskim pregledom, mentalni poremećaj je dijagnostikovan kod 70,8% ispitanika: poremećaji ličnosti (41%), zavisnost od alkohola (8,4%), neurotski poremećaji (6,5%), shizofrene psihoze (5,2%), poremećaji raspoloženja (3,2%), paranoidna psihoza (2,6%), organski poremećaji (1,9%), zavisnost od droga (1,3%) i mentalna retardacija (0,6%). Među poremećajima ličnosti dominira emocionalno-nestabilni (55,6%). Ustanovljeno je da je tokom ubistva kod 77,9% počinilaca bila smanjena uračunljivost, kod većine od njih bitno, a svi kod kojih je dijagnostikovan psihotični poremećaj su proglašeni neuračunljivim. **Zaključak.** Među počiniocima ubistva koji su sudsko-psihijatrijski veštačeni, najčešće su dijagnostikovani emocionalno nestabilni poremećaji ličnosti. Relativno manja zastupljenost psihotičnih poremećaja nameće potrebu za destigmatizacijom, posebno pacijenata koji boluju od teških duševnih oboljenja.

Ključne reči: ubistva; nasilje; forenzička psihijatrija; mentalni poremećaji; poremećaji ličnosti; psihotični poremećaji; socioekonomski faktori; kriminal; alkoholizam; psihička uračunljivost

Corresponding Author: Dr Zoran Gajić, Klinički centar Vojvodine, Klinika za psihijatriju, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: jmilatovic981@gmail.com

Abbreviations

PD – personality disorder
ICD – International Statistical Classification of Disease

Introduction

In addition to being the centre of attention in the field of criminology, forensic psychiatry and forensic psychology for decades, murder as the most serious criminal act also represents a phenomenon of wider social significance. According to the United Nations Office on Drugs and Crime (UNODC) data, 468,000 murders were committed in 2010, 5% of which in Europe [1].

In terms of psychology and psychiatry, murder is the result of a dynamic interactive relationship between the victim, perpetrator of the criminal act and a constellation of factors. Risk factors for committing violent crimes can roughly be divided into two groups: individual factors (biological, personal, sociodemographic, clinical) and environmental factors.

Although numerous risk factors have been registered, the link between mental disorders and homicide has been in the focus of attention for centuries. Regardless of the methodological limitations, studies have shown that there is a growing correlation between violence and mental illness in recent decades [2]. Results of the studies performed in 2004 indicate that over 90% of the homicide offenders have a psychiatric diagnosis, but that only in certain cases of mental disorders there is a higher risk of violence and violent crimes [3].

Individuals suffering from schizophrenia, paranoid psychosis and manic depression are more prone to violence than individuals diagnosed with other mental disorders [4]. Results obtained in the retrospective study clearly show a tenfold higher probability of homicidal behaviour among individuals diagnosed with schizophrenia, regardless of gender [5], while another study has established that the risk of homicide among males is 6.5 times higher than in the general population [6]. Auditory hallucinations and persecutory delusions increase the risk of violent behaviour and can be connected to the motive for homicide [7].

Personality disorder is one of the most frequent diagnoses among those who commit homicide [8]. The prevalence of personality disorders in homicide is high and according to some studies it ranges between 34% [5] and 54% [3]. The most common types are antisocial, narcissistic and borderline personality disorders [9–11].

The authors have found a significant connection between homicide and alcohol consumption [12]. Recent studies have shown that alcohol abuse is a significant risk factor for homicide [13] and violence in general [14]. Not only does alcohol consumption lead to an increase in the number of homicides, but there is also a difference between alcohol-related and non-alcohol-related homicides [15–17].

This study presents sociodemographic and psychiatric characteristics of homicide offenders in the north region of Serbia over a 10 year period (1996-2005).

Material and Methods

This retrospective study includes 154 homicides committed on the territory of the Province of Vojvodina, Serbia in the period between 1996 and 2005. The data were obtained from performed forensic and psychiatric expert investigations and divided into the following categories: sociodemographic data, personal history (including past psychiatric history, substance abuse history, past history of violence), and current psychiatric history (including diagnoses, alcohol or drug abuse preceding the offense). Data were analyzed using the statistical program John's Macintosh programe (JMP) (Perform basic analysis and graphing: Distribution analysis, use a bar chart to visualize the distribution of categorical variable, test probabilities (Pearson), H_O – there is no difference between the groups). The p-value below the significance level of 0.05 (alpha) indicated a statistical significance of the difference.

Results

The homicide offenders were predominately male, with a statistically significant difference (142 out of 154, i.e. 92.2%). The female offenders were older than men, their respective average age being 41.4 and 35.8.

Out of 90 subjects (58.4%, that being more than a half of the total study sample of 154 offenders) who were not married, 69 (44.8%) had never been married, 18 (11.7%) were divorced and 3 (1.9%) were widowed.

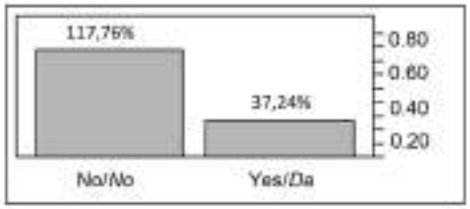
More than half of the subjects had no children (51.9%, n=80/154) or employment (59%, n=91/154), and their level of education was low (87%, n=134/154).

A positive history of criminal acts was found only in one-fourth of the subjects **(Graph 1)**, most of which were theft crimes (41%, n=15/37), while only 5 subjects committed violent crimes. Before they committed homicide, 16 offenders had received a jail sentence**.**

With regard to alcohol use, slightly less than half of the subjects consumed alcohol occasionally (48%, n=74/154)**,** while slightly more than one fifth of them consumed alcohol on a daily basis **(Graph 2).**

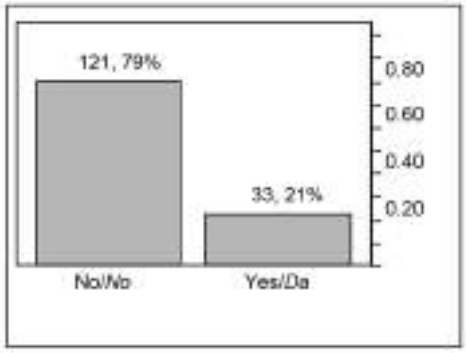
At the time of committing the crimes, more than a half of offenders were under the influence of alcohol (57%, n=88/154), advanced intoxication having been determined in two thirds of them **(Graph 3)**. The great majority of subjects (97.4%, n=150/154) were not under the influence of other psychoactive substances at the time of committing the crimes.

As for the previous psychiatric diagnoses and treatment, the results show that the majority of the subjects never received psychiatric treatment (68%, n=104/154). Upon examining homicide offenders who did undergo psychiatric treatment, it was de-



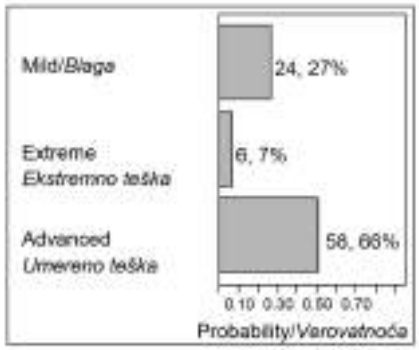
Test	Chi square	DF	Prob./Verovatnoća>Chi Sq none
Pearson	41.5584	1	<.0001*

Graph 1. Positive history of criminal
Grafikon 1. *Prethodne kriminalne aktivnosti*



Test	Chi Square	DF	Prob./Verovatnoća>Chi Sq none
Pearson	50.2857	1	<.0001*

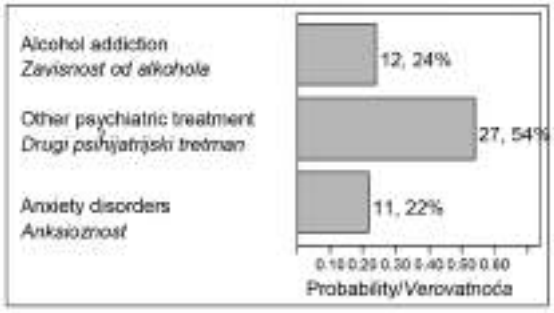
Graph 2. Consumed alcohol on a daily basis
Grafikon 2. *Svakodnevno konzumiranje alkohola*



Test	Chi Square	DF	Prob. (Verovatnoća) > Chi Sq none
Pearson	47.5455	2	<.0001*

Graph 3. Level of intoxication in group of offenders who were under the influence of alcohol
Grafikon 3. *Nivo intoksiciranosti u grupi počinilaca koji su bili pod dejstvom alkohola*

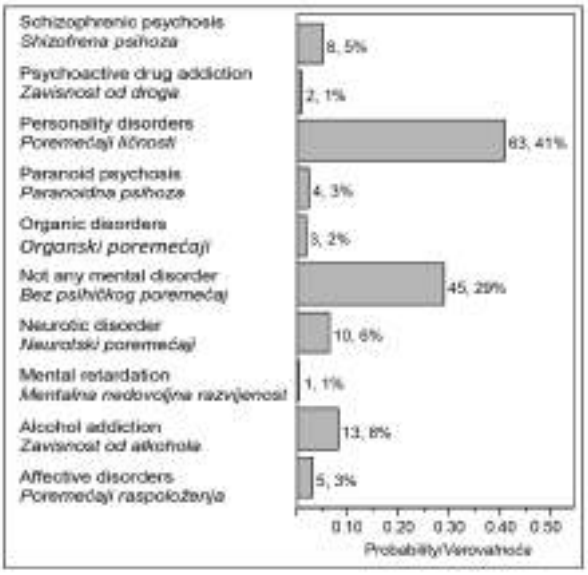
termined that the most frequent diagnoses were alcohol addiction and anxiety disorders **(Graph 4)**



Test	Chi Square	DF	Prob. (Verovatnoća) >Chi Sq none
Pearson	9.6400	2	0.0081*

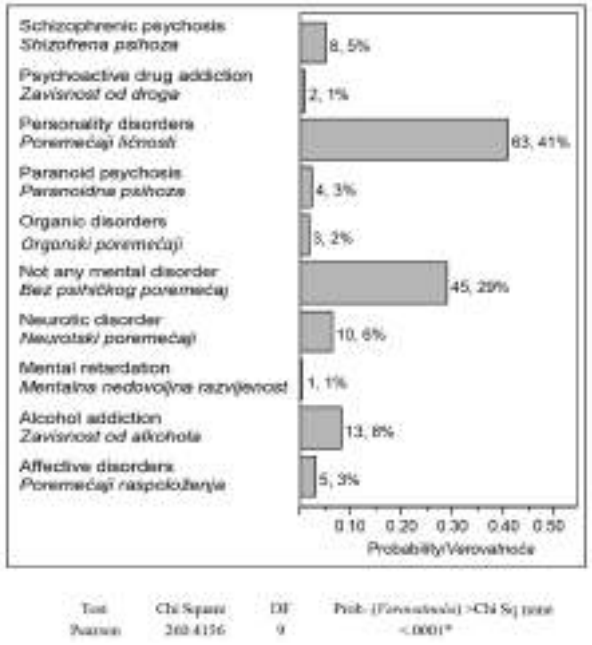
Graph 4. Diagnoses among offenders who previously received psychiatric treatment
Grafikon 4. *Dijagnoze počinilaca koji su prethodno bili u psihijatrijskom tretmanu*

During the psychiatric examination performed for the purpose of forensic expertise, significant majority of subjects were diagnosed with mental disorder (71%, n=109/154). **Graph 5** gives the categories of mental disorders among the diagnosed subjects, showing that personality disorders were dominant, followed by alcohol addiction, neurotic disorders, schizophrenic psychosis, affective disorders, paranoid psychosis, organic disorders, psychoactive drug addiction and mental retardation.



Test	Chi Square	DF	Prob. (Verovatnoća) >Chi Sq none
Pearson	260.4156	9	<.0001*

Graph 5. Categories of mental disorders diagnosed by psychiatric examination
Grafikon 5. *Kategorije mentalnih poremećaja dijagnostikovanih psihijatrijskim veštačenjem*



Graph 6. Assessment of mental competency at the time of homicide
Grafikon 6. *Procena psihičke uračunljivosti tokom izvršenja ubistva*

In the group diagnosed with personality disorders, emotionally unstable personality disorder was dominant, with both its types, impulsive (38%, n=24/63) and borderline one (17.5%, n=11/63).

With regard to the assessment of mental competency at the time of the homicide, diminished mental competency was established in most subjects. All subjects diagnosed with a psychotic disorder were mentally incompetent **(Graph 6).**

Discussion

Bearing in mind the ten-year length of study as well as its specific objective to establish psychiatric characteristics of homicide offenders, it can be said that this is a unique study performed so far on the territory of this region of Serbia. The results of the study contribute to the de-stigmatization of major mental illnesses. The greatest drawback of this study is that data were collected retrospectively, which implies a potential lack of certain information.

We would like to point out our reasoning for the study sample, particularly regarding the time frame (1996-2005). This period in the social circumstances in Serbia and the Balkans had considerable impact on the characteristics of homicide. Namely, the world forensic literature pays special attention to the factors that characterize homicide as predominantly rational as opposed to predominantly affective. In their daily professional work at the Department of Psychiatry in the field of psychiatric expertise of homicide offenses the authors have noticed that the number of homicide has shifted towards predominantly rational homicide in the period of study design.

From the sociodemographic aspect, homicide offenders appeared most likely to be single males, with low level of education, their average age being 36. Compared to studies on homicide published in international literature, the results were similar for half of the homicide offenders – the majority of homicides were committed by subjects of the male gender, while cases of female offenders ranged from 10% to 15%.

Studies have shown the existence of specific circumstances in cases of domestic violence in which the women were the victims [18, 19]. Another distinct characteristic is the fact that women commit infanticide more often than man [20, 21].

In comparison with the results of other studies, in which younger homicide offenders are dominant, the male homicide offenders are older on average in our sample, while the average age of women does not differ from other studies. This may be due to cultural differences and changes in the age structure of homicide offenders over time, which has been contemplated by other authors as well [22, 23].

With regard to the history of violence and previously committed crimes, we came to an unexpected result: in majority of the cases violent crimes had not been registered, nor was there any information on prior aggressive behaviour. Such a result implies that predicting the risk of future dangerousness is complex in spite of the fact that the history of violence and criminal history have been identified as risk factors of violent crimes and are represented in most assessments of violent behaviour [24, 25].

Over half of this study sample subjects were under the influence of alcohol at the time of the homicide, which is in accordance with the results of other studies [26–29]. In this study, only a small number of subjects were under the influence of psychoactive substances at the time of homicide, which can be explained by the fact that drug abuse was significantly lower in this region of Serbia in the period covered by this study compared to the period that followed.

The presence of more serious mental illnesses among the homicide offenders is similar to other international studies. Recent estimates of the rates of psychotic illnesses among those charged with homicide offences are 8. 5% in Finland (5), 10% in England (30), 9. 5% in Ireland (31), 8. 7% in New Zealand (32) and 20% in Sweden (33) and Denmark (34).

The obtained results indicate that only a small number of individuals diagnosed with psychotic disorders are prone to homicidal behaviour.

Based on psychiatric testimonies, which are included in this study, it was determined that all homicide offenders diagnosed with psychotic disorders were mentally incompetent with regard to the criminal act. Criminal law of the Republic of Serbia declares mental incompetency when an individual is not able to grasp the significance of his/her act

or could not control his/her actions due to mental illness, temporary mental disorders, mental retardation or other more serious mental disorders, and such individuals are not subject to penal measures but rather medical measures of safety [35].

Compared to the small number of homicide offenders with psychotic disorders, the number of diagnosed personality disorders is significantly high. Although many studies have proved a correlation between personality disorders and violent crimes [36], the following must also be taken into consideration when evaluating the risk of future dangerousness:

– Most individuals with personality disorders are not prone to violence

– Abnormal personalities are more propitious victims than delinquents

– No personality disorder is necessarily associated with violent behaviour permanently

– From a dynamic and motivational position, personality disorder alone cannot explain violent behaviour [37].

In this study, based on types of personality disorder (PD) according to the International Statistical Classification of Diseases and Related Health Problems (ICD 10) [38], the most common PD is the emotionally unstable personality disorder, the impulsive type being more common than the borderline one. Characteristics like impulsiveness, tendency towards unpredictable behaviour, poor self-control, strong tendency towards conflict and outbursts lead these individuals to violent behaviour.

Dissocial personality disorder (ICD 10) takes the third place in our study sample subjects. Violent behaviour associated with this PD is characterized by its early, stable, versatile onset (applied in different contexts) and frequency within the group. This group is characterized by low empathy, intolerance to frustration, irresponsibility and disregard for authority, disregard for social norms of behaviour, inability to experience guilt and tendency towards blaming others.

The difference between homicide offenders with PD and homicide offenders with psychotic disorders is reflected in the fact that psychotic homicide offenders can be reincorporated into society once they begin taking medication and cease to be a threat to others, while this is not the case with PD, especially in individuals with marked psychopathy, because enduring maladaptive patterns of behaviour, cognition and emotion are to be dealt with in PD.

In addition to using risk assessment to predict aggressive behaviour and dangerousness, which is only a supplementary means, a thorough psychological and psychiatric forensic assessment is necessary in order to prevent recurrence.

Conclusion

This study shows predominant sociodemographic characteristics of homicide offenders: male gender, lower levels of education, single marital status, while younger age and earlier history of violent behaviour have not been substantiated. With regard to psychiatric disorders in cases of homicide offenders who underwent forensic evaluation, emotionally unstable disorders were the most common. A relatively low presence of psychotic disorders implies the need for destigmatization of those patients suffering from major mental illnesses. Stigmatization of psychotic patients in this context is the result of the way the homicides were executed, in other words, due to elements of bizarreness, unexpectedness and impulsivity, all of which arouse reactions such as lack of understanding and fear felt by the society.

References

1. United Nations Office on Drugs and Crime. The 2011 Global Study on Homicide. Vienna: UNODC; 2011.

2. Bradley K. The Bradley Report: Lord Bradley's Review of People with Mental Health Problems or Learning Disabilities in the Criminal Justice System. London: Department of health. 2009.

3. Fazel S, Grann M. Psychiatric morbidity among homicide offenders: a Swedish population study. Am J Psychiatry. 2004;161:2129-31.

4. Hodgins S, Mednick SA. Mental disorder and crime. Evidence from a Danish birth cohort. Arch Gen Psychiatry. 1996;53:489-96.

5. Eronen M, Tiihonen J, Hakola P. Schizophrenia and homicidal behavior. Schizophr Bull. 1996;22:83-9.

6. Tiihonen J, Hakola P. Homicide and mental disorders. Psychiatria Fennica. 1995;26:125-9.

7. Junginger J. Psychosis and violence: the case for a content analysis of psychotic experience. Schizophr Bull. 1966;22:91-103.

8. Flynn S, Rodway C, Appleby L, Shaw J. Serious violence by people with mental illness: national clinical survey. J Interpers Violence. 2014;29:1438-58.

9. Coid J. Personality disorders in prisoners and their motivation for dangerous and disruptive behavior. Crim Behav Ment Health. 2002:209-26.

10. Hodgins S, Cote G. The criminality of mentally disordered offenders. Crim Just Behav. 1993;28:115-29.

11. Rasmussen K, Levander S. Symptoms and personality characteristics of patients in a maximum security unit. Int J Law Psychiatry. 1995;18:117-27.

12. Bye E. Alcohol and homicide in eastern Europe. Homicide studies. 2008;12:7-26.

13. Teplin LA, Jakubowski JA, Abram KM, Olson ND, Stokes ML, Welty LJ. Firearm homicide and other causes of death in delinquents: a 16-year prospective study. Pediatrics. 2014;134:63-73.

14. Wolf A, Gray R, Fazel S. Violence as a public health problem: an ecological study of 169 countries. Soc Sci Med. 2014;104:220-7.

15. Pridmere WAE. A comparison of victim, offender, and event characteristics of alcohol and non-alcohol-related homicides. J Res Crime Delinq. 2008;45:227-55.

16. Shaw J, Hunt I, Flyn S, Amos T, Meehan J, Robinson J, et al. The role of alcohol and drugs in homicides in England and Wales. Addiction. 2006;101:1117-24.

17. Richard-Devantoy S, Bouyer-Richard AI, Jollant F, Mondoloni A, Voyer M, Senon JL. Homicide schizophrenia and substace abuse: a complex interaction. Rev Epidemiol Sante Publique. 2013;61(4):339-50.

18. Bacon W, Lansdowne R. Women who kill husbands: the battered wife on trial. In: O'Donell CC, editor. Family Violence in Australia. Sydney: Longman Cheshir. 1986. p. 67-93.

19. Pereira ARV, Magalhães T. Fatal intimate partner violence against women in Portugal: a forensic medical national study. J Forensic Leg Med. 2013;20:1099-107.

20. Duntley JD, Buss DM. Homicide adaptations. Aggression and Violent Behavior. 2011;16:399-410.

21. Bätje C, Schläfke D, Nedopil N, Hässler F. Infanticide. Social and forensic aspects. Nervenartz. 2011;82:873-9.

22. Paciotti BM. Age and Homicide: Evidence for Persisting Self-help «Honor» Institutions among White Southerners. Davis: University of California and Davis. 2002.

23. Byard RW, Austin AE, van den Heuvel C. Characteristics of asphyxial deaths in adolescence. J Forensic Leg Med. 2011; 18:107-9.

24. Soothill K, Francis B, Ackerley E , Fligelstone R. Murder and Serious Sexual Assault: What criminal histories can reveal about future serious offending. In: Singer L, editor. Policing and Reducing Crime Unit: Police Research Series. London: Pety France; 2002.

25. Kesic D, Thomas SD. Do prior histories of violence and mental disorders impact on violent behaviour during encounters with police? Int J Law Psychiatry. 2014;37:409-14.

26. Dearden J, Payne J. Alcohol and homicide in Australia. Trends and issues in crime and criminal justice. 2009;327:361-80.

27. Dobash RE, Dobash RP, Cavanagh L, Lewis R. Not an ordinary killer-just an ordinary guy: when men murder an intimate woman partner. Violence Against Women. 2004;10:577-605.

28. Dobash RP, Dobash RE, Cavanagh L, Smith D, Medina-Ariza J. Onset of offending and life course among men convicted of murder. Homicide Studies. 2007;11:243-71.

29. Ramsoomar L, Morojele NK. Trends in alcohol prevalence, age of initiation and association with alcohol-related harm among South African youth: implications for policy. S Afr Med J. 2012;102:609-12.

30. Shaw J, Hunt IM, Flynn S. Rates of mental disorder in people convicted of homicide. National clinical survey. Br J Psychiatry. 2006;188:143-7.

31. Dooley E. Homicide in Ireland 1972-1991. Doublin; 1995.

32. Simpson AIF, McKenna B, Skipworth J, Barry-Walsh J . Homicide and mental illness in New Zealand 1970-2000. Br J Psychiatry. 2004;185:394-8.

33. Fazel S, Grann M. Psychiatric morbidity among homicide offenders: a Swedish population study. Am J Psychiatry. 2004;161:2129-31.

34. Gottlieb P, Gebrielsen G, Kramp P. Psychotic homicides in Copenhagen from 1953 to 1983. Acta Psychiatr Scand. 1987;76: 285-92.

35. Krivični Zakonik Republike Srbije. U: Službeni glasnik RS. Beograd. 2010. str. 69-74.

36. Short V, Lennox C, Stevenson C, Senior J, Shaw J. Mental Illness, Personality Disorder and Violence: A Scoping Review 2012. Available from: http://www.ohrn.nhs.uk

37. Esbec E, Echeburua E. Violence and personality disorders: clinical and forensic implications. Acta Esp Psiquiatr. 2010;38:249-61.

38. International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007. Geneva: World Health Organisation; 2007.

Rad je primljen 7. VII 2015.
Recenziran 25. VIII 2015.
Prihvaćen za štampu 10. III 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:224-229.

Clinical Center of Niš, Department of Gynecology and Obstetrics[1]
University of Niš, Faculty of Medicine[2]
Clinical Center of Vojvodina, Novi Sad, Department of Gynecology and Obstetrics[3]
University of Novi Sad, Faculty of Medicine[4]
Clinical Center of Niš
Department of Ophthalmology[5]
Department of Radiology[6]

Original study
Originalni naučni rad
UDK 618.177-089.888.11-036:613.25
DOI: 10.2298/MPNS1608230T

INFLUENCE OF BODY MASS INDEX ON *IN VITRO* FERTILIZATION OUTCOME IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

UTICAJ INDEKSA TELESNE MASE NA ISHOD VANTELESNOG OPLOĐENJA KOD PACIJENTKINJA SA SINDROMOM POLICISTIČNIH JAJNIKA

Milan TRENKIĆ[1], Jasmina POPOVIĆ[1,2], Artur BJELICA[3,4], Vesna KOPITOVIĆ[3,4], Marija TRENKIĆ BOŽINOVIĆ[2,5] and Aleksandra ARACKI TRENKIĆ[6]

Summary

Introduction. The purpose of this study was to investigate the influence of the body mass index on the outcome of in vitro fertilization in patients with polycystic ovary syndrome. **Material and Methods.** The study sample consisted of 123 patients with polycystic ovary syndrome who completed their *in vitro* fertilization treatment at the Department of Gynecology and Obstetrics, Clinical Center Nis, Republic of Serbia, and they were retrospectively analyzed. The patients were divided by body mass index into two groups for the comparison of the findings. One group (normal weight) consisted of women with body mass index ≤ 25 kg/m^2 (mean 22.08±1.90), and the other group (overweight) included women with body mass index > 25 kg/m^2 (mean 27.65±1.47). The patients underwent either the standard long gonadotrophin-releasing hormone agonist protocol or flexible multidose gonadotrophin-releasing hormone antagonist protocol. **Results**. The normal-weight patients had a higher number of mature oocytes, significantly higher fertilization rate (p<0.001) and significantly higher number of the obtained embryos class I (p<0.01) than the overweight patients. However, the implantation rate and clinical pregnancy rate were the same in both groups. **Conclusion**. In the group of overweight women the numbers of mature oocytes and good quality embryos were lower. However, since this study dealt with the patients with polycystic ovary syndrome who generally had a higher number of the obtained oocytes and embryos, this shortfall did not affect clinical pregnancy rates, which were the same in both groups. Certainly, before starting the in vitro fertilization, each infertile patient should be informed about the possible negative effect of her high body mass index on the treatment outcome.

Key words: Body Mass Index; Fertilization *in Vitro*; Treatment Outcome; Polycystic Ovary Syndrome; Embryonic Development; Overweight; Infertility, Female

Sažetak

Uvod. Cilj ove studije bio je da se ispita uticaj indeksa telesne mase na ishod vantelesnog oplođenja kod pacijentkinja sa sindromom policističnih jajnika. **Materijal i metode.** Retrospektivno su analizirane 123 pacijentkinje sa sindromom policističnih jajnika koje su završile postupak vantelesnog oplođenja na Klinici za ginekologiju i akušerstvo Kliničkog centra u Nišu, Republika Srbija. Pacijentkinje su bile podeljene po indeksu telesne mase u dve grupe. Jednu grupu (normalno uhranjene) činile su žene sa indeksom telesne mase ≤ 25 kg/m^2 (prosečan 22,08 ± 1,90), a drugu grupu (prekomerno uhranjene) činile su žene sa indeksom telesne mase > 25 kg/m^2 (prosečan 27,65 ± 1,47). Pacijentkinje su bile tretirane standardnim dugim protokolom agonistima oslobađajućeg hormona za gonadotropine ili fleksibilnim, višedoznim protokolom antagonistima oslobađajućeg hormona za gonadotropine. **Rezultati.** Normalno uhranjene pacijentkinje su imale veći broj zrelih jajnih ćelija, značajno veću stopu oplodnje (p < 0,001) i značajno veći broj dobijenih embriona klase I (p < 0,01) u odnosu na prekomerno uhranjene pacijentkinje. Međutim, stope implantacije i stope kliničkih trudnoća bile su iste u obe grupe. **Zaključak.** U grupi prekomerno uhranjenih žena, broj zrelih jajnih ćelija i broj kvalitetnih embriona bio je niži. Međutim, kako je ova studija rađena kod žena sa sindromom policističnih jajnika koje generalno imaju veći broj jajnih ćelija i embriona, ova razlika nije uticala na stopu kliničkih trudnoća, koje su bile iste u obe grupe. Svakako, pre početka vantelesnog oplođenja, svaka infertilna pacijentkinja treba da bude informisana o mogućem negativnom uticaju njenog visokog indeksa telesne mase na ishod lečenja.

Ključne reči: indeks telesne mase; *in vitro* fertilizacija; ishod lečenja; sindrom policističnih jajnika; razvoj embriona; gojaznost; ženska neplodnost

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects approximately 5–10% of women of reproductive age [1, 2]. Certain metabolic changes including obesity, insulin resistance, and type 2 diabetes have been associated with this syndrome [3–5]. PCOS is the most commonly en-

Corresponding Author: Dr Milan Trenkić, Klinički centar of Niš, Klinika za ginekologiju i akuserstvo, 18000 Niš, Bulevar dr Zorana Đinđića 48, E-mail: trenkic@gmail.com

Abbreviations

BMI – body mass index
PCOS – polycystic ovary syndrome
IVF – *in vitro* fertilization
GnRH – gonadotrophin-releasing hormone
FSH – follicle stimulating hormone
ET – embryo transfer
ICSI – intracytoplasmic sperm injection
hCG – human chorionic gonadotropin
OHSS – ovarian hyperstimulation syndrome

countered cause of anovulatory infertility in patients seeking fertility treatment [6]. For women diagnosed with PCOS and refractory to conventional infertility treatment or having coexisting infertility factors, *in vitro* fertilization (IVF) and embryo transfer (ET) are considered to be an effective treatment modality [7, 8]. Obesity and overweight are commonly linked to PCOS. Moreover, obesity increases the risk of pregnancy complications [9–11].

More than half of reproductive-aged women are overweight, their body mass index (BMI) being 25–29.9 kg/m^2 or obese, having the BMI >30 kg/m^2 [12, 13]. Women with an increased BMI in comparison to those with normal BMI (18.5–24.9 kg/m^2) are at a three times higher risk of infertility caused by disorders of the hypothalamic–pituitary axis, menstrual cycle irregularities and anovulation [14, 15].

However, there are conflicting reports about the influence of an increased BMI rate on the quality of oocytes, lower number of mature oocytes, embryo development and lower implantation and pregnancy rates in assisted reproduction [16, 17]. The effects of BMI on IVF outcomes are also known in the non-PCOS population [18, 19]. Although the improvement of fertility due to weight loss has been demonstrated in PCOS patients attempting spontaneous conception [20–22], the research studies performed on PCOS so far have not yet completely assessed the effect of BMI on the outcomes of IVF [16, 23–26].

In their review from 2007, Maheshwari et al. [16] reported lower pregnancy rates after IVF, along with the requirements for higher doses of gonadotropins for achieving a sufficient ovarian response, as well as higher miscarriage rates among obese and overweight women having a BMI of 25 kg/m^2 or higher. However, they concluded that there was not sufficient evidence for drawing a reliable conclusion on the influence of BMI on cycle cancellation, oocyte recovery, and live birth. In a systematic review on the influence of BMI and IVF-ET outcomes, Rittenberg et al. [27] stated that clinical pregnancy and live birth rates were lower, whereas miscarriage rates were higher in women with BMI ≥ 25 kg/m^2 undergoing IVF/intracytoplasmic sperm injection (ICSI). On the other hand, certain reports suggested that there were no significant differences in the IVF/ICSI outcomes between obese and non-obese PCOS patients [1, 25]. This could be caused by different cut-off points for BMI and by the differences between inclusion criteria used in the studies and/or varying focus of outcome measures.

Clinical outcomes such as pregnancy or implantation rate, rather than the quality of oocytes and embryos, have been the main point of interest of most of the studies dealing with the influence of obesity on infertility. The aim of this study was to examine the influence of BMI in women with PCOS on the outcomes of IVF under the assumption that a poorer oocyte and embryo quality in women undergoing IVF are associated with increased BMI.

Material and Methods

A retrospective analysis included data from the computer database of the Department of Gynecology and Obstetrics, Clinical Center Nis, Republic of Serbia on PCOS patients undergoing IVF treatment in the period between June 2013 and December 2014. An ethics committee approval was granted for the study. All of the patients gave their written informed consent for the participation. The study included exclusively the patients with PCOS who fulfilled the criteria established by the recent ESHRE/ASRM Consensus (2004), i.e. who met any two of the three criteria after exclusion of other causes: oligoovulation and/or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries detected by ultrasound [28]. The study sample consisted of 123 patients diagnosed with PCOS. The additional criteria for the inclusion were: age 18-39 years, BMI of 18-30 kg/m², the value of basal hormonal follicle stimulating hormone (FSH) levels in the early follicular phase <12 IU/L. The exclusion criteria were the following: abnormalities of the uterine cavity, the presence of an uncontrolled thyroid disease, surgically diagnosed endometrioses, submucosal myoma, ovarian cysts discovered on transvaginal ultrasound and the existence of severe spermatogenetic disturbances in the patients' partners that required ICSI technique. The patients were divided into two groups according to their BMI. The normal weight group included women with BMI ≤ 25 kg/m^2 while women with BMI >25 kg/m^2 formed the overweight group. Each patient could participate in the study only once.

The patients were treated by either standard long gonadotropin-releasing hormone (GnRH) agonist protocol or flexible multidose GnRH antagonist protocol. The GnRH agonist long protocol treatment included daily administration of 0.1 mg triptorelin (Diphereline, Ipsen Pharma Biotech, France), started on day 21 of the previous menstrual cycle and continued until the administration of human chorionic gonadotropin (hCG). Following the confirmation of down-regulation after 13-15 days (assessed by the serum estradiol levels <20 pg/mL, serum luteinizing hormone (LH) <2.0 mIU/mL, and no ovarian cysts present), gonadotropin stimulation was performed. The antagonist protocol, consisting of daily gonadotropin stimulation, was started on day 2 or 3 of menstruation. When the leading follicle reached the size of 14 mm and/or the levels of estradiol reached the value >300 pg/mL, a daily injection of cetrorelix

0.25 mg (Cetrotide; Merc Serono, Switzerland) was included. The treatment was continued until the day of hCG injection. A recombinant FSH (Gonal F, Merc Serono, Switzerland) was used in both protocols for gonadotrophin stimulation. The decision on the protocol used for ovarian stimulation was made on the basis of patients' characteristics and previous response in the IVF cycles.

The starting FSH dose, injected subcutaneously, was individually adjusted according to the ovarian response evaluated by transvaginal ultrasound assessment and measuring the serum estradiol levels. The moment when three follicles reached the mean diameter of ≥17 mm or when the dominant follicle measured ≥18 mm and the following two ≥16 mm, a dose of 10.000 IU hCG (Pregnyl, Organon, Holland) was administered intramuscularly in both protocols. At 34-36 h after the hCG administration, transvaginal ultrasound-guided oocyte retrieval was performed.

The retrieved oocytes that reached the metaphase II were classified as mature (MII), whereas those that reached metaphase I (MI) or germinal vesicle (GV) stage were considered as immature. Using a conventional IVF method, insemination was performed 38-40 hours after the hCG administration. Two pronuclei (PN) appearing 16-18 hours following the insemination confirmed normal fertilization. The fertilization rate was expressed as the number of zygotes with two pronuclei over the total number of inseminated oocytes. The embryo transfer was performed under transabdominal ultrasound guidance. The embryos were transferred on day 3. A daily dose of vaginal progesterone (Utrogestan 600 mg/day; Laboratories Besins-International S.A., France) was used for the luteal phase support and a serum pregnancy test was performed 12 days after the embryo transfer. The intrauterine gestational sac and fetal cardiac activity visualized by transvaginal ultrasound at 6-7 weeks of gestation confirmed clinical pregnancy. A clinical pregnancy loss prior to the 20 weeks of gestation was considered a miscarriage.

The embryo scoring was performed in accordance with the internal laboratory embryo score standards. Morphological features such as equal or unequal size of blastomeres and the presence or absence of fragmentation of cytoplasm, as well as the dynamics of embryo development, were used as parameters for the evaluation of the embryo quality. The dynamics of embryo development was performed by monitoring the number of blastomeres every 24 hours until the day of ET and by comparison of the actual number of blastomeres with their expected number. According to the above parameters, the embryo grading system consisting of four classes of embryos was created. The embryos assigned to the class I on day 3 or 68±1h after the insemination satisfied all of the following three criteria: 1. the embryos had 6 to 8 blastomeres; 2. the blastomeres were equal; 3. the blastomeres had no fragmentation. The class II embryos did not meet one of the above three criteria – they either had less than 6 blastomeres or had 6-8 blastomeres but of an unequal size, or there was fragmentation of the blastomeres. The embryos of the class III did not fulfill two of the criteria and the embryos belonging to the class IV did not satisfy any of the stated criteria.

A modified classification system based on combined criteria reported by Golan et al. [29] was used to determine the grades of severity of ovarian hyperstimulation syndrome (OHSS). The patients having the symptoms of mild OHSS such as abdominal distension and discomfort were classified as Grade I. Other symptoms of mild OHSS included nausea, vomiting, diarrhea, as well as an ovarian enlargement of 5-12 cm. The features of mild OHSS in combination with the ascites detected by ultrasound were considered as moderate OHSS or Grade II. The patients requiring hospitalization due to the development of a severe or critical OHSS, or because of their medical condition fulfilled one or more of the hospital admission criteria, were included in Grade III or severe OHSS. The hospital admission criteria required the presence of one of the following features: ascites, hydrothorax, hematocrit ≥45%, oliguria, elevated liver enzymes, dyspnoea, anasarca or acute renal failure.

The primary outcome measures included the number of mature oocytes, fertilization rate, and quality of embryos. The secondary outcome measures consisted of the total gonadotropin dose requirements, clinical pregnancy rate, implantation rate, miscarriage rate, and the incidence of OHSS.

The continuous variables were described by means±standard deviations and by medians. For the categorical variables, absolute numbers and percentages were given. The distributions of the continuous variables were assessed for normality by the Shapiro-Wilk test. The differences between independent groups were analyzed by an unpaired t-test in case of a normal distribution or by Mann-Whitney test if the distribution of data was not normal. A chi-square test was used to compare proportions of categorical variables between groups. The level of significance was set at 0.05. The calculations were carried out using the SPSS statistical package version 15.0.

Results

A total of 123 patients with PCOS who fulfilled the specified inclusion criteria were analyzed. The patients were divided into two groups: 96 women in the group with normal weight, with a BMI ≤ 25 kg/m^2 and 27 in the overweight group, with the BMI >25 kg /m^2. The average age of the patients, the average BMI in the groups, the number of cycles, and the rate of the applied protocols are given in **Table 1**.

The total amount of gonadotropins used for ovarian stimulation, as well as the duration of stimulation did not differ between the two groups. The proportion of mature oocytes in the total number of aspirated oocytes was statistically higher in the women with normal weight (70.89% vs. 64.84%). In addition, the fertilization rate was statistically higher in the pati-

Table 1. Patients' characteristics and treatment regimen
Tabela 1. *Karakteristike pacijentkinja i vrsta primenjenog protokola stimulacije*

Variable/*Promenjive*	BMI ≤25 kg/m^2	BMI >25 kg/m^2
No. of patients/*Br. pacijentkinja*	96	27
Mean BMI (kg/m^2)/*Prosečan BMI (kg/m^2)*	22.08±1.90 (22.00)	27.65±1.47 *** (28.00)
Patients' age (years)/*Starost pacijentkinje (godine*)	31.65±3.99 (32.00)	31.59±4.35 (31.00)
No. of cycles/*Br. ciklusa*	96	27
Protocol type/*Tip protokola*		
Long GnRH agonist/*Dugi GnRH- agonisti*	60 (62.50%)	18 (66.67%)
Flexible GnRH antagonist/*Flexibilni GnRH- antagonisti*	36 (37.50%)	9 (33.33%)

*** – p<0.001 Mann-Whitney test, Unpaired Student t-test/Chi square tests,
GnRH - gonadotropin-rilizing hormon, BMI - indeks telesne mase

Table 2. Ovarian stimulation characteristics
Tabela 2. *Karakteristike ovarijalne stimulacije*

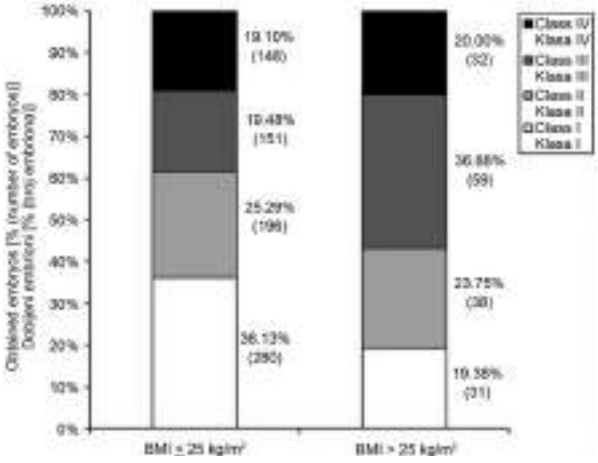
Variable/*Promenjive*	BMI ≤25 kg/m^2	BMI >25 kg/m^2
Total gonadotropin dose/*Ukupna doza gonadotropina (IU)*	1599.09 ± 474.09 (1550.00)	1759.72 ± 685.81 (1575.00)
Duration of stimulation (days)/*Dužina stimulacije (dani)*	10.29 ± 2.24 (10.00)	10.63 ± 2.20 (10.00)
The total number of oocytes retrieved *Ukupan broj preuzetih oocita*	1350	310
Mean number of retrieved oocytes *Srednji broj preuzetih oocita*	14.06 ± 7.89 (13.50)	11.48 ± 6.52 (11.00)
The total number of retrieved mature oocytes *Ukupan broj preuzetih zrelih oocita*	957 (70.89%) *	201 (64.84%)
Mean number of mature oocytes/*Srednji broj zrelih oocita*	10.07 ± 6.33 (9.00)	7.44 ± 4.86 (6.00)
Fertilization rate (%)/*Stopa oplodnje*	68.22% (921/1350)***	57.10% (177/310)
The total number of obtained embryos *Ukupan broj dobijenih embriona*	775	160
Mean number of obtained embryos *Srednji broj dobijenih embriona*	8.42 ± 4.90 (7.00)	6.40 ± 3.96 (5.00)
Total number of obtained embryos class I *Ukupan broj dobijenih embriona klase I*	280 (36.13%)***	31 (19.38%)
Mean number of obtained embryos class I *Srednji broj dobijenih embriona klase I*	3.04 ± 3.10 (2.00)**	1.24 ± 1.74 (1.00)
Endometrial thickness on hCG day (mm) *Debljina endometrijuma na dan davanja hCG-a (mm)*	10.39 ± 1.46 (10.10)	9.89 ± 1.57 (10.00)

* – p<0.05, ** – p<0.01, *** – p<0.001 Mann-Whitney test, Unpaired Student t-test, Chi square tests
hCG - humani horionski gonadotropin, BMI - indeks telesne mase

Table 3. Pregnancy outcome and complications
Tabela 3. *Ishod trudnoće i komplikacije*

Variable/*Promenjive*	BMI ≤25 kg/m^2	BMI >25 kg/m^2
No. of embryos transferred/*Broj transferiranih embriona*	2.70 ± 0.61 (3.00)	2.80 ± 0.50 (3.00)
Implantation rate/*Stopa implantacije*	24.19%	22.86%
No. of clinical pregnancies per ET/*Broj kliničkih trudnoća po ET*	45/92 (48.91%)	12/25 (48.00%)
No. of biochemical pregnancies per ET/*Broj biohemijskih trudnoća po ET*	3/92 (3.26%)	0/25 (0.00%)
No. of multiple pregnancies per ET/*Broj multifetalnih trudnoća po ET*	10/92 (10.87%)	1/12 (8.33%)
No. of miscarriages/*Broj pobačaja*	7/45 (15.56%)	1/12 (8.33%)
No. of cancelled cycles/*Broj otkazanih ciklusa*	4 (4.17%)	2 (7.41%)
OHSS	15 (15.63%)	2 (7.41%)
Grade I/*Gradus I*	14 (14.58%)	1 (3.70%)
Grade II/*Gradus II*	1 (1.04%)	1 (3.70%)

Legend/*Legenda*: ET - embryo transfer/*embrio transfer;* OHSS - ovarian hyperstimulation syndrome/*sindrom ovarijalne hiperstimulacije*



Graph 1. The effect of BMI on embryo quality. Distribution of the obtained embryos by classes on day 3
Grafikon 1. *Uticaj indeksa telesne mase na kvalitet embriona. Distribucija dobijenih embriona po klasama trećeg dana*

ents with BMI ≤ 25 kg/m^2 (68.22% vs. 57.10%). The presence of class I embryos in the total number of the obtained embryos (36.13% vs. 19.38%), and their average number was statistically higher in the normal weight women group compared to the overweight ones. These data are shown in **Table 2**.

The proportions of the obtained embryos classified by the quality in relation to the BMI are shown in **Graph 1**.

Table 3 shows the pregnancy outcomes and complications. The implantation rate, the rate of clinical, biochemical and multifetal pregnancies were similar. However, the incidence of miscarriages and OHSS was higher in the normal weight women, but it was not statistically significant.

Discussion

There is a close relation between obesity and PCOS, as well as certain overlapping features [30]. In addition, the association between these two disorders is related to insulin resistance. The BMI is known to be in oppostie correlation with the response to some drugs and decrease in the body weight contribute to a better reproductive outcome [31]. It has been determined that less gonadotropin ampoules for stimulation as well as a higher number of the obtained oocytes and embryos are required for women with PCOS undergoing IVF. Furthermore, they are also more prone to OHSS. However, no agreement has yet been reached regarding the influence of BMI on the IVF outcome. Although a poorer IVF outcome has been reported by most of the studies, a certain number of them did not associate the poorer outcome with overweight (BMI: 25–29.9 kg/m^2), but only with obesity (BMI > 30 kg/m^2).

Our study did not show any statistically significant difference between the normal and the overweight group related to the total dose of gonadotropins. As it has been previously established, a larger amount of gonadotropins is required to stimulate obese patients. The association with the volume of distribution or peripheral metabolic clearance may be a possible reason for gonadotropin resistance in these patients [32]. This discrepancy can be explained by the fact that the median BMI among women of the overweight group was 28 kg/m^2 and that the women over BMI of 30 kg/m^2 could not undergo IVF before losing weight.

The same number of oocytes, but a lower total number of mature oocytes was obtained in the group of normal weight patients, as well as the same number of the obtained embryos, but a smaller number of those belonging to class I. Such a result could be only related to the BMI since the median age in both groups was 31.

The precise mechanism of how BMI influences the reproductive outcome is still unclear. A low embryo quality can be possibly caused by adverse follicular conditions related to insulin resistance, endocrine alterations, and possibly, by embryo toxic cytokines [23, 25, 33, 34]. The levels of estrogen and androgen are modified by obesity. The impairment of folliculogenesis and follicular atresia are caused by this endocrine disturbance due to an increased secretion of luteinizing hormone [35], an increased ratio of androgen [36], hyperinsulinemia [37] and an increased production of insulin-like growth factor (IGF-1) [38]. Another possible mechanism is a potential influence of adipokines leptin, adiponectin, ghrelin PYY3-36 and resistin on energy homeostasis, all of which can affect female fertility [39, 40].

In our study, there was no statistically significant difference in the IVF outcome between the groups. The number of the obtained mature oocytes and class I embryos was smaller in the group of overweight patents. However, the implantation rate and clinical pregnancy rate were the same. The mean number of aspirated oocytes was 11 in the overweight group and the mean number of obtained embryos was 5. Due to such a high number of embryos we were able to choose the best quality ones for transfer.

Conclusion

Female overweight is associated with a fewer number of mature oocytes and fewer good quality embryos; however, since the number of the obtained oocytes and embryos in polycystic ovary syndrome patients included in this study was generally higher, this shortfall did not affect pregnancy rates, which were the same in both groups. Certainly, before starting the *in vitro* fertilization, each infertile patient should be informed about the possible negative effect of the high body mass index on the treatment outcome. Furthermore, higher body mass index increases the risk of complications during pregnancy and at childbirth.

References

1. Beydoun HA, Stadtmauer L, Beydoun MA, Russell H, Zhao Y, Oehninger S. Polycystic ovary syndrome, body mass index and outcomes of assisted reproductive technologies. Reprod Biomed Online. 2009;18(6):856-63.

2. Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Alexopoulou E, et al. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: a prospective randomised controlled trial (RCT). Hum Reprod. 2010;25(3):683-9.

3. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev. 1997;18(6):774-800.

4. Fedorcsak P, Dale PO, Storeng R, Tanbo T, Abyholm T. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. Hum Reprod. 2001;16(6):1086-91.

5. Barber TM, McCarthy MI, Franks S, Wass JA. Metabolic syndrome in polycystic ovary syndrome. Endokrynol Pol. 2007;58(1):34-41.

6. Franks S. Polycystic ovary syndrome. N Engl J Med. 1995;333(13):853-61.

7. Buyalos RP, Lee CT. Polycystic ovary syndrome: pathophysiology and outcome with *in vitro* fertilization. Fertil Steril. 1996;65(1):1-10.

8. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89(3):505-22.

9. Linne Y. Effects of obesity on women's reproduction and complications during pregnancy. Obes Rev. 2004;5(3):137-43.

10. Stothard K, Tennant P, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA. 2009;301(6):636-50.

11. Metwally M, Saravelos S, Ledger WL, Li TC. Body mass index and risk of miscarriage in women with recurrent miscarriage. Fertil Steril. 2010;94(1):290-5.

12. Balen AH, Anderson RA; Policy and Practice Committee of the BFS. Impact of obesity on female reproductive health: British Fertility Society, policy and practice guidelines. Hum Fertil. 2007;10(4):195-206.

13. Koning AMH, Kuchenbecker WKH, Groen H, Hoek A, Land JA, Khan KS, at al. Economic consequences of overweight and obesity in infertility: a framework for evaluating the costs and outcomes of fertility care. Hum Reprod Update. 2010; 16(3):246-54.

14. Van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Michgelsen HW, et al. Predictive value of pregnancy history in subfertile couples: results from a nationwide cohort study in the Netherlands. Fertil Steril. 2008;90(3):521-7.

15. Brewer CJ, Balen AH. The adverse effects of obesity on conception and implantation. Reproduction. 2010;140(3):347-64.

16. Maheshwari A, Stofberg L, Bhattacharya S. Effect of overweight and obesity on assisted reproductive technology-a systematic review. Hum Reprod Update. 2007;13(5):433-44.

17. Bellver J, Ayllón Y, Ferrando M, Melo M, Gori E, Pellicer A, et al. Female obesity impairs in vitro fertilization outcome without affecting embryo quality. Fertil Steril. 2010;93(2):447-54.

18. Moragianni VA, Jones SM, Ryley DA. The effect of body mass index on the outcomes of first assisted reproductive technology cycles. Fertil Steril. 2012;98(1):102-8.

19. Shah DK, Missmer SA, Berry KF, Racowsky C, Ginsburg ES. Effect of obesity on oocyte and embryo quality in women undergoing *in vitro* fertilization. Obstet Gynecol. 2011;118(1):63-70.

20. Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. Hum Reprod. 2003;18(9):1928-32.

21. Hollmann M, Runnebaum B, Gerhard I. Effects of weight loss on the hormonal profile in obese, infertile women. Hum Reprod. 1996;11(9):1884-91.

22. Tolino A, Gambardella V, Caccavale C, D'Ettore A, Giannotti F, D'Antò V, et al. Evaluation of ovarian functionality after a dietary treatment in obese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2005;119(1):87-93.

23. Jungheim ES, Lanzendorf SE, Odem RR, Moley KH, Chang AS, Ratts VS. Morbid obesity is associated with lower clinical pregnancy rates after *in vitro* fertilization in women with polycystic ovary syndrome. Fertil Steril. 2009;92(1):256-61.

24. Marquard KL, Stephens SM, Jungheim ES, Ratts VS, Odem RR, Lanzendorf S, et al. Polycystic ovary syndrome and maternal obesity affect oocyte size in *in vitro* fertilization/intracytoplasmic sperm injection cycles. Fertil Steril. 2011;95(6):2146-9.

25. McCormick B, Thomas M, Maxwell R, Williams D, Aubuchon M. Effects of polycystic ovary syndrome on *in vitro* fertilization-embryo transfer outcomes are influenced by body mass index. Fertil Steril. 2008;90(6):2304-9.

26. Luke B, Brown MB, Stern JE, Missmer SA, Fujimoto VY, Leach R; SART Writing Group. Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates. Hum Reprod. 2011;26(1):245-52.

27. Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis. Reprod Biomed Online. 2011;23(4):421-39.

28. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.

29. Golan A, Ron -el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. Obstet Gynecol Surv. 1989;44(6):430-40.

30. Cırık DA, Dilbaz B. What do we know about metabolic syndrome in adolescents with PCOS? J Turk Ger Gynecol Assoc. 2014;15(1):49-55.

31. Bjelica A, Bjelanović J, Milić N, Segedi Lj, Ilić Đ, Dimitrijević J. Algoritam indukcije ovulacije kod pacijentkinja sa sindromom policističnih jajnika. Med Pregl. 2016;69(1-2):25-30.

32. Akpınar F, Demir B, Dilbaz S, Kaplanoğlu I, Dilbaz B. Obesity is not associated with the poor pregnancy outcome following intracytoplasmic sperm injection in womenwith polycystic ovary syndrome. J Turk Ger Gynecol Assoc. 2014;15(3):144-8.

33. Metwally M, Cutting R, Tipton A, Skull J, Ledger WL, Li TC. Effect of increased body mass index on oocyte and embryo quality in IVF patients. Reprod Biomed Online. 2007;15(5):532-8.

34. Carrell DT, Jones KP, Peterson CM, Aoki V, Emery BR, Campbell BR. Body mass index is inversely related to intrafollicular HCG concentrations, embryo quality and IVF outcome. Reprod Biomed Online. 2001;3(2):109-11.

35. Balen AH. Hypersecretion of luteinizing hormone and the polycystic ovary syndrome. Hum Reprod. 1993;8 Suppl 2:123-8.

36. Hsueh AJ, Billig H, Tsafriri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr Rev. 1994; 15(6):707-24.

37. Poretsky L, Grigorescu F, Seibel M, Moses AC, Flier JS. Distribution and characterization of insulin and insulin-like growth factor I receptors in normal human ovary. J Clin Endocrinol Metab. 1985;61(4):728-34.

38. Adashi EY, Resnick CE, D'Ercole AJ, Svoboda ME, Van Wyk JJ. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr Rev. 1985; 6(3):400-20.

39. Mitchell M, Armstrong DT, Robker RL, Norman RJ. Adipokines: implications for female fertility and obesity. Reproduction. 2005;130(5):583-97.

40. Budak E, Ferna´ndez Sa´nchez M, Bellver J, Cervero´ A, Simo´n C, Pellicer A. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. Fertil Steril. 2006;85(6):1563-81.

Rad je primljen 24. V 2016.
Recenziran 13. VI 2016.
Prihvaćen za štampu 14. VI 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:230-236.

CASE REPORTS
PRIKAZI SLUČAJEVA

Institute for Cardiovascular Diseases of Vojvodina, Sremska Kamenica
Department of Cardiology[1]
University of Novi Sad, Novi Sad, Faculty of Medicine[2]
Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica
Department of Urgent Pulmonology[3]

Case report
Prikaz slučaja
UDK 616.12-008.3-083.98:621.38
DOI: 10.2298/MPNS1608237I

FIRST APPLICATION OF AUTOMATED EXTERNAL DEFIBRILATOR IN SERBIA – CASE REPORT

PRVA PRIMENA AUTOMATSKOG SPOLJAŠNJEG DEFIBRILATORA U SRBIJI − PRIKAZ SLUČAJA

Igor IVANOV[1,2], Jadranka DEJANOVIĆ[1,2], Milenko ČANKOVIĆ[1,2], Vladimir IVANOVIĆ[1,2], Aleksandra VULIN[1,2] and Dušanka OBRADOVIĆ[2,3]

Summary

Introduction. Sudden cardiac death is an unexpected natural death from cardiac causes. It is the most common and first manifestation of coronary artery disease. It accounts for 50% of mortality from cardiovascular disease in the United States of America and other developed countries, so measures that can reduce it are an important medical task. **Case Report.** A 55-year old man suddenly lost consciousness at the train station in Novi Sad. An eyewitness provided first aid and ventricular fibrillation was converted to sinus rhythm by means of the automated external defibrillator. Emergency Medical Service Novi Sad soon arrived, continued resuscitation procedure, and transported the patient to the Cardiac Care Unit, who was then diagnosed with acute myocardial infarction and primary percutaneous coronary intervention was performed. Resuscitative hypothermia was applied in acute phase to prevent further brain injury. During further hospitalization the patient was stable, woke up from coma and early rehabilitation measures were implemented. After six months the patient had normal physical activities and there was no left ventricular segmental hypokinesia on echocardiography. **Conclusion.** The application of all four chains of survival is important in increasing the survival rate of patients with sudden cardiac arrest.

Key words: Defibrillators; Serbia; Death, Sudden, Cardiac; Ventricular Fibrillation; Myocardial Infarction; First Aid; Cardiopulmonary Resuscitation; Hypothermia, Induced; Percutaneous Coronary Intervention; Health Education; Treatment Outcome

Sažetak

Uvod. Iznenadna srčana smrt predstavlja neočekivanu prirodnu smrt srčanog uzroka. Najčešća je i često prva manifestacija koronarne bolesti. Odgovorna je za oko 50% mortaliteta od kardiovaskularne bolesti u Sjedinjenim Američkim Državama i drugim razvijenim zemljama. Primena mera koje je mogu smanjiti predstavljaju značajan medicinski zadatak. **Prikaz slučaja.** Muškarac star 55 godina iznenada je izgubio svest na železničkoj stanici u Novom Sadu. Očevici su pružili prvu medicinsku pomoć i primenili automatski spoljašni defibrillator koji je registrovao ventrikularnu fibrilaciju i isporučio defibrilaciju nakon čega se uspostavio sinusni ritam. Ekipa službe hitne medicinske pomoći iz Novog Sada brzo je stigla na mesto događaja i nastavila primenu mera resuscitacije nakon čega je bolesnika transportovala u jedinicu intenzivne kardiološke nege. Dijagnostikovan je akutni infarkt miokarda nakon čega je učinjena primarna perkutana koronarna intervencija. U akutnoj fazi primenjena je postresuscitativna hipotermija da bi se sprečilo dalje moždano oštećenje. Tokom dalje hospitalizcije bolesnik je bio stabilan. Probudio se iz kome, nakon čega su primenjene mere rane rehabilitacije. Posle šest meseci bolesnik je normalno fizički aktivan, a ehokardiografski se nije registrovao ispad segmentne kinetike koji je bio prisutan u akutnoj fazi. **Zaključak.** Primena sva četiri lanca preživljavanja je važna u povećanju postotka preživaljavanja bolesnika sa iznenadnim srčanim zastojem.

Ključne reči: defibrilatori; srbija; iznenadna srčana smrt; ventrikularna fibrilacija; infarkt miokarda; prva pomoć; kardiopulmonalna resuscitacija; indukovana hipotermija; perkutana koronarna intervencija; zdravstveno vaspitanje; ishod lečenja

Introduction

Sudden cardiac death (SCD) is an unexpected natural death from cardiac causes within a short time interval, typically within one hour of the onset of symptoms, in persons with no history of conditions which could seem fatal [1]. Coronary artery disease is the most common cause of SCD [2]. SCD is the most common and often the first manifestation of coronary artery disease and is responsible for about 50% of mortality from cardiovascular disease in the United States of America and other developed countries [1].

Corresponding Author: Prof. dr Igor Ivanov, Institut za kardiovaskularne bolesti Vojvodine, Klinika za kardiologiju, 21204 Sremska Kamenica, Put dr Goldmana 4, E-mail: igor.ivanov@ikvbv.ns.ac.rs

Abbreviations

SCD	– sudden cardiac death
AED	– automated external defibrillator
ICU	– Intensive Care Unit
ECG	– electrocardiogram
EF	– ejection fraction
VF	– ventricullar fibrilation
PCI	– percutaneous coronary intervention

The epidemiology of coronary artery disease is parallel to SCD, as 80% of persons with SCD have a coronary artery disease [1]. Prevention of SCD is done by preventing risk factors that may lead to it. Another very important task is to reduce morbidity following the establishment of circulation and respiration. These measures must include all types and levels of health care, as well as paramedics and eyewitnesses. In this paper we present a case of a patient who survived SCD caused by ventricular fibrillation based on ischemic heart disease. He survived thanks to well-organized care and respecting all current recommendations for the treatment of SCD and acute myocardial infarction.

Case Report

A 55-year old man suddenly lost consciousness at the train station in Novi Sad. A Serbian Railway worker provided first aid using the automated external defibrillator (AED) which registered the ventricular fibrillation, and applied an electrical shock in order to convert it to sinus rhythm. In the meantime, an eyewitness called the Emergency Medical Service Novi Sad, which soon arrived and continued the resuscitation procedure. Because of insufficient breathing, the patient was intubated, sedated, mechanically ventilated, and transported to the Intensive Care Unit (ICU) at the Department of Cardiology, Institute for Cardiovascular Diseases of Vojvodina, where all measures of cardiopulmonary resuscitation were applied. Upon admission to the ICU, the patient was unconscious, Glasgow Coma Score (GCS) 3, endotracheal intubated, artificially ventilated, hypotensive, 90/60 mmHg, normal frequency of 75/min, without signs of heart failure. The patient was analgo-sedated and placed on invasive mechanical ventilation in intermittent positive pressure ventilation (IPPV) mode. Invasive hemodynamic monitoring was also applied. Initial medical therapy for acute myocardial infarction was given and because of hemodynamic instability, inotropes were included. The electrocardiogram (ECG) done on admission registered signs of acute myocardial infarction (AMI) of anterolateral region **(Figure 1)**.

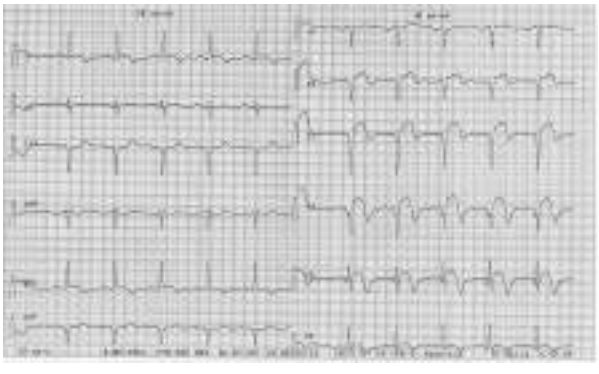


Figure 1. Electrocardiogram on admission
Slika 1. *Elektrokardiogram na prijemu*

Echocardiographic examination registered initially dilated left ventricle with hypokinesa of anteroseptal segments, hypertrophic myocardium, ejection fraction (EF) of 45% with mild mitral and aortic regurgitation. On the basis of clinical presentation, ECG and echocardiogram, the diagnosis of acute myocardial infarction was set, and urgent coronary angiography was indicated. Coronarography revealed an occlusion in medial segment of the left anterior descending coronary artery (RIA), stenosis of the first diagonal artery (D1) and 95% stenosis of the proximal segment of the dominant right coronary artery (ACD). Primary percutaneous coronary intervention (pPCI) was performed with stent implantation in the medial segment of the left anterior descending and proximal right coronary artery with an optimal result with TIMI 3 flow **(Figures 2 and 3)**.

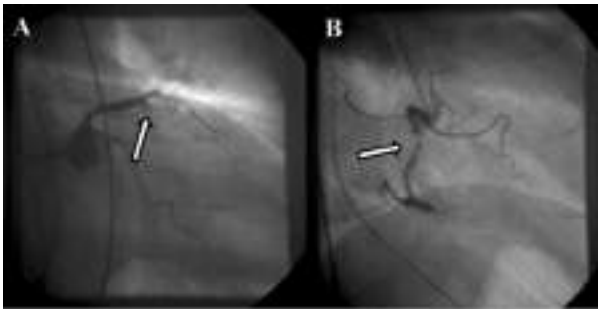


Figure 2. The finding of occlusion of the medial segment of the RIA (arrow) and significant changes in the medial segment of the ACD (right)
Slika 2. *Okluzija mdijalnog segmenta prednje međukomorne gradne (RIA) (strelica) i signifikantne promene medijalnog segmenta desne koronarne arterije (desno)*

Coma resulted from the cardiac arrest and in order to prevent further brain damage resuscitative hypothermia was applied with special external cooling blankets and cold infusion solutions according to the International Liaison Committee on Resuscitation (ILCOR) protocol [3]. For intravascular application, solutions cooled at 4 °C were used at a dose of 30 ml/kg at a rate of 100 ml/min. Body temperature was maintained in the range of 33–34 °C for 24 hours with continued sedation. The hemodynamic variables were observed with invasive monitoring. The following values were achieved: mean arterial pressure of 90 mmHg, central venous pressure of about 5 mmHg, pulmonary capillary "wedge" of around 12 mmHg with electrocardiographic monitoring which registered rare premature ventricular beats. Inotropic stimulation was gradually interrupted. On the second

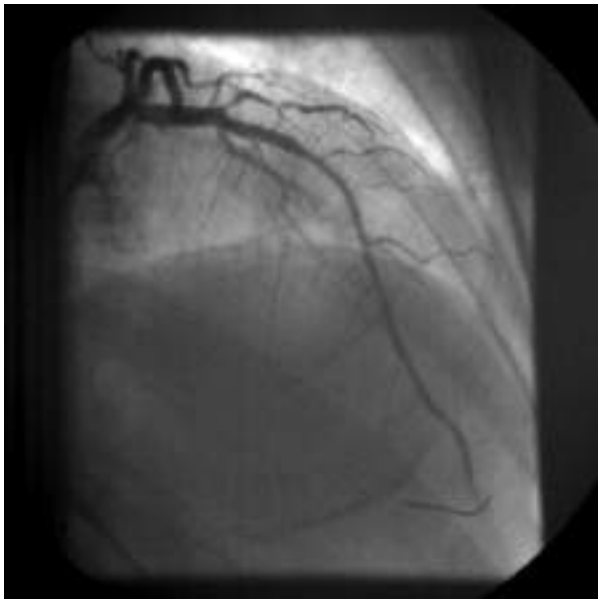


Figure 3. Recanalization of RIA
Slika 3. *Rekanalizacija prednje međukomorne grane (RIA)*

day the protocol of resuscitation hypothermia was discontinued, the patient spontaneously heated to 0.5 °C/h, and became sub-febrile to 37.6 °C, which was the reason for introducing broad spectrum antibiotic treatment and parenteral cephalosporin of the third generation. On the third day of hospitalization, the patient was extubated with short-term psychomotor agitation and somnolence. In the further course some improvements developed and he was conscious, oriented and mentally inconspicuous until his discharge. There were no neurological disorders. While being in the ICU, the patient was compensated, normocardic and normotensive without inotropic stimulation, subfebrile to 37.8 °C. The patient underwent early rehabilitation measures. On the sixth day of hospitalization, he was transferred from the ICU to the ward to continue further treatment. Ultrasound findings of the carotid artery indicate insignificant changes in the carotid arteries with fibrolipid plaques. Throughout hospitalization, the patient remained without any subjective complaints, he was hemodynamically stable, and on the 15th day of hospitalization the patient was discharged to continue outpatient treatment.

After one month the check-up showed that the patient was physically active and without subjective complaints. After six months, the patient remained without subjective complaints, and echocardiography that was performed showed preserved systolic function of the left ventricle, EF 56%. Stress ECG was without any signs of ischemia and lesions.

Discussion

European Resuscitation Council has defined four rings of survival in patients with cardiac arrest [2]. The first ring in the chain of survival represents the prevention of SCD, or identification of patients at risk and procedures aimed for prevention. The next ring is timely and proper cardiopulmonary resuscitation, followed by early defibrillation. Application of publicly accessible defibrillators (PAD) is justified in areas with a large number of people and the possibility of a great incident. Thus, setting an AED has its own purpose in areas where the incidence of cardiac arrest is such that it is used once within five years [4]. This corresponds to approximately one sudden cardiac arrest in 1,000 cases per year. These devices have a role in environments where the arrival of emergency service is not possible within five minutes [4]. There is also a need to work on educating the wider community and employees at these places, who would be able to recognize and begin cardiopulmonary resuscitation and use the AED. The first condition in achieving full recovery of patients after cardiac arrest is to establish spontaneous rhythm. Ventricular fibrillation (VF) is the most common initial rhythm of SCD. The sooner you achieve defibrillation, the prognosis is better. The survival rate after VF cardiac arrest declines by around 7–10% every minute of delayed defibrillation [5].

In our case, placing AEDs in public places such as railway stations proved to be justified. Cardiac arrest was immediately recognized by passers-by, and this first aid reaction was made successful by means of the AED with fast defibrillation. The establishment of spontaneous breathing and circulation was followed by post-resuscitation care which included actions aimed at resolving the post cardiac arrest syndrome. Coronary heart disease is the most common disease among patients with hospital cardiac arrest, and the treatment of acute myocardial infarction is necessary to reduce morbidity after cardiac arrest. Recommendations of the European Society of Cardiology (ESC) induce percutaneous coronary intervention (PCI) within 120 minutes of first medical contact. The centers which are able to do primary PCI are those centers where this procedure is carried out 24/7 all year round and those able to start the procedure as soon as possible and within 60 minutes of arrival at the facility [6].

In our patient, the primary PCI was performed within the expected time. Due to the successful recanalization of, several coronary arteries, in this case, the preserved contractile function of the left ventricular myocardium was registered on control visits. Since the brain, being the organ which is most vulnerable to the lack of oxygen, is the first to be damaged after cardiac arrest, these patients often have neurological disability despite the establishment of circulation and respiration. Therapeutic hypothermia has been recognized in the latest guidelines of the European Resuscitation Council (ERC) in comatose patients as a method that can help protect the brain and contribute to reducing morbidity. The role of hypothermia is recognized in patients with initial non-shockable, as well as shockable rhythms [2].

The method of hypothermia was introduced at our Institute in 2005, and since then has been routinely used in patients after cardiac arrest. A study from our

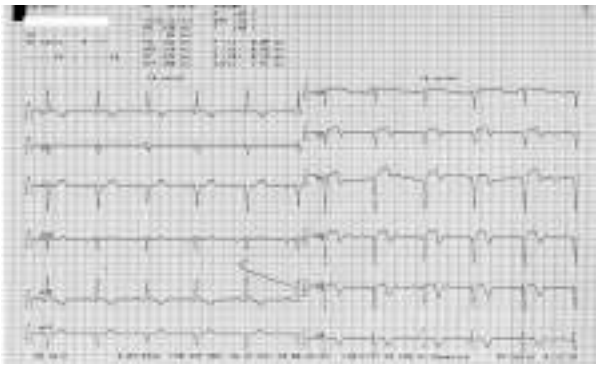


Figure 4. ECG at discharge
Slika 4. *Elektrokardiogram na otpustu*

Department showed a clear benefit from the use of such methods [7]. The application of early hypothermia in our patients was achieved after the first day of confusional state with complete recovery and without residual neurological deficits. Post-resuscitation care, primarily pPCI and early therapeutic hypothermia prevented the complications of acute myocardial infarction and avoided a disability in this patient. In this paper, we present all available measures to address acute myocardial infarction and its most common lethal complication of ventricular fibrillation with a positive outcome. The patient shortly returned to his normal activities despite severe illness.

Conclusion

Well functioning of all rings in the chain of survival requires community involvement and cooperation at all levels of health care. This is a lengthy and difficult task, but each success brings new life and reduces morbidity. In our case, we have shown how it is practically possible with a positive outcome.

References

1. Zipes DP, Wellens HJJ. Sudden Cardiac Death. Circulation. 1998;98:2334-51.

2. Deakin CD, Nolan JP, Soar J, Sunde K, Koster RW, Smith DE, et al. European Resuscitation Council Guidelines for Resuscitation 2010 Section 4. Adult advanced life support. Resuscitation. 2011;82(1):140th.

3. Nolan JP, Morley PT, Vanden Hoek TL. Therapeutic hypothermia after cardiac arrest. An advisory statement by the Advance Life Support Task Force of the International Liaison Committee on Resuscitation. Resuscitation. 2003;57:231-5.

4. ECC Guidelines Part 4: The Automated External Defibrillator Key Link in the Chain of Survival Circulation. 2000; 102:160-76.

5. Larsen MP, Eisenberg MS, Cummins RO, Hallstrom AP. Predicting survival from out-of-hospital cardiac arrest: a graphic model. N Engl J Med. 1993;22:1652-8.

6. Task Force Members: Steg G, James S, Atar D, Badano L, Blo¨mstrom-Lundqvist C, Borger M, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. European Heart Journal 2012;33:2569-619.

7. Petrovic M, Srdanović I, Panić G, Čanji T, Miljević T. Our experience in applying resuscitativne hypothermia. Med Pregl. 2007;60(9-10):431-5.

Rad je primljen 3. II 2016.
Recenziran 18. V 2016.
Prihvaćen za štampu 26. V 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:237-240.

SEMINAR FOR PHISICIANS
SEMINAR ZA LEKARE U PRAKSI

Clinical Center of Vojvodina, Novi Sad
Department of Obstetrics and Gynecology[1]
University of Novi Sad, Faculty of Medicine[2]

Seminar for physicians
Seminar za lekare u praksi
UDK 618.177-089.888.11:612.64.085.2
DOI: 10.2298/MPNS1608241B

ASSESSMENT OF THE EMBRYO QUALITY IN THE PROCEDURE OF *IN VITRO* FERTILIZATION

PROCENA KVALITETA EMBRIONA U POSTUPKU IN VITRO FERTILIZACIJE

Artur BJELICA[1,2] and Srna SUBANOVIĆ[1]

Summary

Introduction. Since reproductive technologies are becoming increasingly popular among the couples with infertility problem, and having in mind that the success rate is still low, the clinicians tend to transfer more embryos in order to increase the probability of success. However, such a strategy increases the risk of multiple pregnancy, which brings about numerous risks to the health of both the mother and children. Therefore, an elective single-embryo transfer is set as imperative, which, on the other hand, would not be possible without selection and evaluation of the quality of embryos. **Assessment of Embryo Quality.** Embryos can be selected by various methods, from non-invasive to invasive methods. In non-invasive methods, the embryos are selected by their morphology or by the techniques based on the analysis of molecular components – analyses of the level of proteomes or metabolomes. A more detailed monitoring of the kinetics of the embryo development can be related to the introduction of time-lapse imaging and monitoring systems into laboratory practice. The invasive methods encompass the techniques such as preimplantation genetic diagnosis and preimplantation genetic screening. In preimplantation genetic diagnosis, the assisted reproduction technologies cycle is approached for the genetic reasons, whereas preimplantation genetic screening is used to enhance the success rate of the assisted reproduction cycles. **Conclusion**. In this paper we have shown that the application of elective single-embryo transfer requires the selection and assessment of the quality of embryos by the methods that have been developed in the last four decades, and still need further improvements.

Key words: Embryonic Development; Fertilization *in Vitro*; Selection, Genetic; Embryo Implantation; Genetic Testing; Preimplantation Diagnosis; Single Embryo Transfer; Pregnancy Rate; Risk Factors; Time-Lapse Imaging; Metabolomics; Proteomics

Sažetak

Uvod. Budući da asistirane reproduktivne tehnologije postaju sve popularnije među parovima koji se leče od neplodnosti, a kako je stopa uspeha i dalje niska, klinike se odlučuju za transfer većeg broja embriona, kako bi tu stopu povećale. Međutim, takva strategija povećava rizik od ostvarivanja multiple trudnoće koja sa sobom nosi brojne rizike, kako po zdravlje majke, tako i po zdravlje dece. Zbog toga se kao imperativ postavlja elektivni transfer jednog embriona koji, bez selekcije i procene kvaliteta embriona, ne bi bio moguć. **Procena kvaliteta embriona.** Embrioni se mogu selekcionisati primenom brojnih metoda – od neinvazivnih do invazivnih. Kod neinvazivnih metoda, embrioni se biraju na osnovu njihove morfologije ili putem tehnika zasnovanih na analizi njihovih molekularnih komponenata – analizi nivoa proteoma ili metaboloma. Detaljnije praćenje kinetike razvoja embriona postiže se primenom laboratorijskih tehnika kontinuiranog nadzora embriona putem sistema monitoringa. Invazivne metode obuhvataju preimplantacionu genetsku dijagnostiku i preimplantacioni genetski skrining. Dok se kod preimplantacione genetskle dijagnostike, asistirana reprodukcija sprovodi radi genetskih ispitivanja, tako se preimplantacioni genetski skrining sprovodi sa ciljem povećanja uspešnosti ciklusa asistirane reprodukcije. **Zaključak**. Ovim radom ukazano je na to da se procena kvaliteta i selekcija embriona za elektivni transfer jednog embriona bazira na primeni brojnih metoda razvijenih u poslednje četiri decenije, ali da ovo polje zahteva nastavak istraživanja i usavršavanja samih tehnika procene.

Ključne reči: razvoj embriona; *in vitro* fertilizacija; genetska selekcija; implantacija embriona; genetsko testiranje; preimplantaciona dijagnoza; transfer jednog embriona; ishod trudnoće; faktori rizika; time-lapse imidžing; metabolomi; proteomi

Introduction

The inability to obtain offspring represents a serious medical, psychological as well as sociological problem, both for an individual and for the partners, so that it is rightly considered as a "partners' problem".

The beginning of *in vitro* fertilization (IVF) dates back to the end of the 19th century, with the experiments on animal models [1]. As early as in 1965, Pro-

Corresponding Author: Prof. dr Artur Bjelica, Klinički centar Vojvodine, Klinika za ginekologiju i akušerstvo, 21000 Novi Sad, Branimira Ćosića 37, E-mail: dr.artur@eunet.rs

Abbreviations

ART – assisted reproduction technologies
ET – embryo transfer
hpi – hours post insemination
ICM – inner cell mass
ICSI – intracytoplasmic sperm injection
IVF – *in vitro* fertilization
PB – polar body
PGD – preimplantation genetic diagnosis
PGS – preimplantation genetic screening
TE – trophoectoderm
ZIFT – zygote intrafallopian transfer
ZP – zona pellucida

fessor Robert G. Edwards et al. attempted to fertilize oocytes *in vitro*, and after 13 years their efforts were crowned with success: on July 25, 1978 Louise Brown was born as the first test-tube baby [1]. Almost three decades later, when millions of children had been born this way, Professor Robert G. Edwards was awarded the Nobel Prize for physiology/medicine in 2010 for the development of IVF [2, 3]. Today, there are more than 2000 clinics involved worldwide, the biggest being the one in Tokyo, which treats more than 15,000 couples for infertility annually [2].

However, the success rates are still low; lower than the ones that would satisfy both the doctors and the patients, and a much bigger problem are high percentages of multiple pregnancies after the assisted reproduction. While physicians consider multiple pregnancies to be a problem, to the patients, due to their great desire to obtain offspring, they seem to be a great success. Because of that, a successful selection of embryos is a necessity in order to decrease the number of transferred embryos, and thus reduce the risk of multiple pregnancy and increase the success rate. Thus a solution would be a selective single-embryo transfer (eSET), the success of which is not possible without the selection and assessment of the quality of the embryo.

Infertility – Basic Notions, Types and Causes

Infertility is defined as the state in which there is no conception after one year of regular sexual intercourse without contraception. The expression "infertility" denotes the incapability of carrying out the pregnancy and giving birth to a viable child, and we should distinguish it from the notion of sterility [4, 5]. Sterility may be primary and secondary. Primary sterility denotes the woman's incapability of conception, whereas secondary sterility refers to the inability of the woman to get pregnant after one previous pregnancy.

Assisted Reproduction Technologies

According to the World Health Organization, assisted reproduction technologies (ART) inlcude the following methods: embryo transfer (ET), intracytoplasmic sperm injection (ICSI), ovarian stimulation with exogenous gonadotropins, surgical laparoscopy, and surrogacy [6]. Besides, the ART methods include also gamet intrafalopian transfer (GIFT), zygote intrafalopian transfer (ZIFT), and frozen embryo transfer (FET), but they are applied to a much lesser extent. In the literature, one can also find preimplantation genetic diagnosis (PGD), gamete and embryo donation, as well as gamete and embryo cryopreservation, mentioned as ART methods. It is thought that approximately 99% of ART cycles are performed by IVF-ET [7].

In IVF, as one of the most known ART method, egg(s) from the woman's ovary are fertilized in laboratory conditions and after several days returned back, i.e. the embryo/s is/are implanted in the uterus of the same or another woman [4].

It is stated in the literature that over three million [4] or even five million [8] children were born worldwide thanks to IVF. In the recent years there has been an increase in the number of IVF newborns, but also an increase in the number of multiple pregnancies. The reason for this can be found in the practice of ovarian stimulation and pregnancy of women of older age, which represents a risk for a multiple pregnancy.

Assessment of Embryo Quality

Morphology is an appropriate marker of viability of the implantation potential of the embryo. Embryologists often describe a morphologically normal embryo as "nice". However, morphology is not esthetics, and we cannot speak of an absolute correlation between the normal morphology and the positive outcome of the IVF treatment. In view of the fact that this assessment is subjective, it has been endeavored for years or even decades to find a way of transforming the embryo assessment into an objective and quantitative method [9, 10]. There are disputes over whether it is necessary to select embryos at all, bearing in mind the possibility of their freezing. However, if we take a look at the data it comes out that the rate of successful implantation is lower after the transfer of defrozen embryos, which would lead to the situation that the success rate could not be raised to a higher level. In addition, from the aspect of the patients who want to decrease the number of visits to the clinics and number of cycles in order to avoid additional costs and lessen emotional stress, embryo selection appears as a necessity.

Embryos can be selected by non-invasive and invasive methods. In non-invasive methods, the embryos are selected according to their morphology or by the techniques based on the analysis of molecular components – analyses at the level of proteomes or meabolomes [11]. Time-lapse imaging has recently been developed. Invasive methods encompass the techniques such as PGD and preimplantation genetic screening (PGS).

Morphology as a Tool for the Assessment of Embryo Quality

The quality of embryos is mainly assessed on the basis of their morphology, i.e. based on the key morphological characteristics that are in correlation with an enhanced implantation rate. Examination of the embryos is carried out under the microscope after taking them from the incubator. Because of the harmful effects

of taking out the embryo from the incubator, the procedure is limited to only several discrete observations, which limits the amount of collected information, and the embryo assessment is highly dependent on the timing of the information [12]. In order to achieve a more reliable assessment of the quality of the embryos, more frequent evaluation would be needed; however, this means also more frequent exposition of the embryo(s) to the changes in temperature, gas components and humidity [13]. When conventional incubators are used, there is a conflict between the need to obtain a detailed picture of the development of embryos and disturbance of the stable conditions of the culture [11]. There are two main approaches to embryo assessment, performed either sequentially in several stages of the embryo development or only once, immediately prior to the transfer [14].

Timing of Observation of Fertilized Oocytes and Embryos

It is accepted that standardization of the time of observation is critical for the possibility of comparison of the results among the laboratories. Assessments are uniformly expressed in hours post insemination (hpi). Thus, if the day of insemination is denoted by 0, then day 5 is the last possible day for embryo transfer.

Assessment of the Quality of Oocytes

In view of the fact that the gamets provide the future embryo with more than two haploid sets of chromosomes, it is clear that the quality of oocytes plays a crucial role in the determination of the development of the embryo and consequently to its viability [15]. A conclusion of the Istanbul Workshop was that optimal morphology of oocytes is a spherical structure surrounded by a uniform zona pellucida (ZP) with a uniform translucent cytoplasm with no inclusions, as well as with the corresponding size of the polar body (PB) [16]. Anomalies of oocytes can be classified as extracytoplasmic and intracytoplasmic dysmorphisms [17]. In the assessment of the quality of oocytes one can carry out scoring of cumulus-oocyte complex (COC), ZP, perivitelline space, PB, vacuolizations, etc. [16, 18].

Assessment of Fertilization

In the assessment of fertilization it is necessary to detect whether the fertilization took place in an appropriate way. It is necessary to analyze pronuclei and nucleolar precursor bodies (NPBs) after 16-18 hpi [18]. Optimal appearance of a fertilized oocyte should be spherical with two PBs and two centrally localized pronuclei. There are different systems of scoring the nuclei, which use different criteria for the prediction of the further development and quality of the embryo [19–21].

Embryos at the Stage of Cleavage

According to the Istanbul consensus, the evaluation of embryos at the stage of cleavage is carried out 26±1 h after ICSI and 28±1 h after IVF [16]. The most common criteria that are used to evaluate the embryo quality at this stage are the number of cells and their morphology [16, 18], percentage of fragmentations [16, 22, 23], but also the cell size [16, 24], multinucleation [16, 25–27], and others. Numerous studies have shown retrospectively which embryos are best, i.e. the ones having the highest implantation potential. Some of the characteristics of such embryos are: 4 or 5 blastomers on day 2 and at least 7 blastomers on day 3 after the fertilization, absence of multinucleated blastomers, and less than 20% of fragmentations on day 2 and day 3 after the fertilization [18]. Furthermore, it has been shown that the frequency of mitotic divisions is associated with the embryo development potential since the cleavage rates, which can be faster and slower than the expected one, are associated with a poorer developmental potential of the embryo [12, 18, 20, 28].

Embryo Evaluation on Day 4 (Morula Stage)

The optimal embryo at the morula stage, i.e. 92±2 h after the insemination, should be compact or in the phase of compaction, and that it entered at the fourth cleavage [16, 29]. The compaction should include the whole embryo.

Embryo Evaluation on Day 5 (blastocyst stage)

Grading of the blasocyst, which is its morphological evaluation, includes the following stages according to the Istanbul Consensus: early, expanding, expanded, hatching or hatched, as well as the quality of inner cell mass (ICM) and trophoectoderm (TE) cells [16]. At this stage as well as at the stage of cleavage, the time of evaluation and morphology plays an important role in the selection of balstocysts for transfer. An optimal embryo at this stage of development, i.e. 116±2 h after insemination, should be a completely expanded blastocyst to the hatched blastocyst, with a pronounced ICM, which is easily discernable. It should have many cells which are in compaction and strongly connected, whereas TE consists of many cells that form a cohesive epithelium [16]. ICM is of great importance for the implantation potential and fetal development, as well as TE, which has been the subject of numerous studies [18, 30, 31]. It should be pointed out that many authors have proposed some other systems for grading of blastocysts as well [18, 32–34].

It has been shown that if the blastocyst collapses in the process of evaluation, it cannot be reliably evaluated [16]. Such blastocysts should be re-evaluated after 1-2 h, bearing in mind that a new expansion of a blastocyst can normally take place even in regular cycles. The time of blastocyst formation is of special importance, which has been confirmed by many studies [18].

Transfer of the Blastocyst

Despite numerous studies and generally accepted opinion that the blastocyst transfer increases the success rate, the published data still show that this hypothesis is not, at least partly, true [31]. The reason for this is that the blastocyst transfer increases the success rate only in the patients with good prognosis, i.e. the patients who have a large number of good-quality embryos on day 3, but only if they want to achieve pregnancy in the fastest way. In addition, the transfer of embryos at the stage of cleavage is

recommended to those women who want to have a cumulative pregnancy rate and possibility of giving birth to all their embryos.

Some authors are of the opinion that the embryos which are of good quality at the stage of cleavage should be transferred on day 3, otherwise they will not survive prolonged culturing to the stage of blastocysts. On the other hand, some researchers are of the opposite opinion, and they think that the embryos which did not reach the stage of blastocyst were not of good quality even at the stage of cleavage.

OMICS Techniques – Noninvasive Techniques

In view of the fact that the evaluation of morphological parameters does not provide information on the embryo physiology, the need for the development of techniques capable of doing that emerged, and therefore OMICS techniques, have been developed.

In order to apply these noninvasive methods, IVF centers have to fulfill several criteria such as the possibility of measuring the changes without damaging the embryo, the ability to perform fast measurement of the changes, and the possibility to carry out measurements in a strictly correct way [18].

Metabolomics and Proteomics

In contrast to genomics and transcriptomics, the analysis of proteins and other metabolites is not an invasive procedure. Besides, its advantage is also that the expendable medium is an excellent source of the material. However, its shortcoming is the low concentration of these components.

Metabolomics

Metabolomics is a new technique which enables measurement of the factors in the embryo culture medium, such as glucose, pyruvate and amino acids, as well as many others. In order to determine the metabolites associated with physiological and pathological states, different spectral (near infrared spectroscopy (NIR) and Raman spectroscopy) and other analytical approaches are applied. Studies of metabolites showed that the metabolic profile of the embryo that results in the pregnancy is different from that of the embryos that do not lead to pregnancy [18, 35]. However, a conclusion has been drawn that the morphology of the embryo is not fully associated with its physiology.

Proteomics

The proteomics profiling is most often performed by mass spectrometry, but also by other ionizing methods that enable precise, fast and cheap analysis of small-volume samples at the sensitivity level of picomols to femtomol. Numerous factors have been studied, such as human leukocyte antigen G (HLA-G), platelet activating factor (PAF), leptin, ubiquitin, etc. However, in spite of the significant development, the knowledge of proteomics of preimplantation embryos is still limited. The reason for this is the limited amount of the sample, low gene expression, and poor sensitivity of the proteomic platform [36]). Of course, one should understand that the development of the embryo depends on many factors and we cannot expect that one factor might be capable of predicting the embryo developmental or implantation potential.

Presently, the investigations are oriented towards non-invasive analyses of preimplantation genetic screening (PGS), resulting in non-invasive screening of the viability, including chromosomal constitution and the discovery of protein lipocalin-1 in the blastocyst secretome. It has been shown that this protein is associated with aneuploidy since its expression was elevated in the secretome samples of the aneuploid blastocysts [37].

Wang [38] mentioned that the process of embryo selection, which once was "competition in beauty", i.e. a simple assessment of the appearance of the embryo will soon include metabolic, proteinic, and genomic markers as the evaluation markers.

Time-Lapse Monitoring

A more detailed monitoring of the kinetics of the embryo development can be related to the introduction of time-lapse imaging (TLI) and monitoring systems into laboratory practice. The continuous monitoring of the progress of the embryo development is possible by following the key indicators of this process (both positive and negative) such as formation of the pronucleus, early cleavage, cell cycle intervals, synchronization of cell division and initiation of blastulation, and multinucleation, etc., since all these contribute to the selection of the best embryos for transfer [11]. This enables the precise determination of the beginning, duration, and time lapse between the cell divisions [39]. Furthermore, it allows getting a detailed insight into the embryo development and studying of the effects of exposure of the embryo to different factors, which can contribute to the improvement of optimal culture conditions, and thus enhance the success rate.

There are two most often used time-lapse systems – Primo Vision (Vitrolife) and Embryoscope (Fertilitech) based on bright field technology, whereas the third one, EEVA (Early Embryonic Viability Assement, Auxogyn) uses dark field [8]. In all the systems the embryos are photographed in the intervals of 5-20 min, and the obtained photos can be joined into a short video. Time-lapse incubator supports the embryo development in the same way as the conventional incubator does [40].

Preimplantation Genetic Diagnostics and Preimplantation Genetic Screening

According to ESHRE, PGD should be distinguished from Preimplantation Genetic Screening (PGS). Namely, in PGD, the ART cycle is approached for the genetic reasons, whereas in PGS genetic screening is applied in order to enhance the success rate of the ART cycle. Besides, the aims are also different: the goal of PGD is to obtain a healthy child, whereas the aim of PGS is to obtain a child.

Both PGD and PGS use the following materials: polar body, blastomere, and trophoectoderm cells. The most frequently applied methods are: FISH (fluorescent in situ hybridization) – for the analysis

of chromosomes, PCR (polymer chain reaction) – for the diagnosis of monogenetic diseases, and CGH (comparative genomic hybridization) – for chromosomal rearrangements. It should be pointed out that the method of detection at the level of a single cell is the same as the method used for other tissues for prenatal diagnosis. Still, it is more difficult to work with one cell since certain difficulties are encountered (cell lysis, allele drop out or some other problems due to mosaicism) [41].

Preimplantation Genetic Screening is used in the infertile couples who at a low risk of transmitting hereditary diseases to the offspring (in contrast to the couples who pass through PGD), because of which it is often termed "low risk PGD" [42].

Ethical and Legal Aspects of ART – Embryo Selection

Besides bringing the benefits to the couples who cannot produce offspring in a normal way, assisted reproduction technologies raise numerous ethical, legal, cultural, and social questions. Great Britain was first to regulate the ART issues [2]. In Arabic countries, there are few acts regulating ART matter, and the majority of them follow the religious laws. In contrast to that, surrogates and donations are allowed in the United States, and there are no limitations of the number of transferred embryos. In Germany, it is permitted to create at most three embryos, and all of them have to be transferred. Therefore, selection of embryos is not permitted, which means that PGD and PGS are illegal. The same holds for Switzerland and Italy. In our country, neither the Orthodox nor Catholic Church accepts IVF fully, i.e. they consider it unethical, especially because of the creation of a number of embryos, so that there is a concern about the fate of the redundant embryos after the completed cycle. The act which regulates ART issues in the Republic of Serbia is the "The Law on Treatment of Infertility by Procedures of Biomedically-assisted Fertilization" [43]. It regulates the issues related to PGD, donation of the gametes and embryos, the number of transferred embryos, and the way of performing the PGD procedure.

In view of the fact that our country is among ten states in the world with oldest population (the share of those over 65 is more than 16.5%) [44], it is obvious that it is necessary to stimulate the partners to become parents. But it is also necessary to help the partners with infertility problems to get acquainted with the methods of assisted reproduction, using different methods and forms of information and education. Because of the lack of information about the risks of multiple pregnancy, and because the state finances only two IVF attempts, the couples decide to transfer more embryos. Hence, it is necessary that our scientific and other system institutions, as well as governmental health-care institutions – the Ministry of Health, social politics, etc. should regularly gather information about modern achievements in the field of assisted reproduction, contribute to its advancement, and support research and more unpaid attempts.

Conclusion

In this work we have shown that the application of elective single embryo transfer requires the selection and assessment of the quality of embryos by the methods that have been developed in the last four decades, and still need further improvements.

It is hoped that these approaches will become a part of routine laboratory practice in the future, and, along with evaluation of embryo morphology parameters, will give a sound base for success in this pursuit. While we are waiting for the development of more precise non-invasive technologies of embryo quality assessment, good laboratory practice and close care in all steps of the in vitro fertilization cycle remain indispensable.

References

1. The history of IVF – The Milestones. [Internet]. [cited 2015 April 29]. Available from: http://www.ivfworldwide.com/ivf-history.html.

2. Coward K, Wells D, editors. Textbook of Clinical Embryology. New York: Cambridge University Press; 2013.

3. Human *in vitro* fertilization, Nobelförmsamlingen, The Nobel Assemblz at Karloinska Institutet. [Internet]. [cited 2015 April 29]. Available from: http://www.nobelprize.org/nobel_prizes/medicine/laureates/2010/.

4. Đurđević S, Kopitović V, Kapamadžija A. Ginekologija. Novi Sad: Univerzitet u Novom Sadu Medicinski fakultet; 2011.

5. Radunović N. Osnove lečenja neplodnosti, Postupci asistirane reprodukciju u lečenju infertiliteta. Acta clinica Vol. 9, No3. Beograd: Klinički centar Srbije; 2009. p. 13-42.

6. Assisted Reproductive Technologies (ARTs). [Internet]. [cited 2015 April 29]. Available from: http://www.who.int/genomics/gender/en/index6.html.

7. Assisted Reproductive Technologies. [Internet]. [cited 2015 April 28]. Available from: http://www.sart.org/SART_Assisted_Reproductive_Technologies.

8. Kovacs P. Embryo selection: the role of time-lapse monitoring. Reprod Biol Endocrinol. 2014;12(124).

9. Centers for Disease Control and Prevention. Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinics Reports. Atlanta, US Department of Health and Human Services; 2010.

10. ESHRE. Embryo selection for IVF, Is there a signal in the noise? Focus on Reproduction. September, 2011.

11. Kirkegaard KK. Human embryo development and viability assessed by time-lapse imaging. [dissertation]: Health Aarhus University; 2013.

12. Montag M, Liebenthron J, Koster M. Which morphological scoring system is relevant in human embryo development? Placenta. 2011;32:252-6.

13. Zhang JQ, Li XL, Peng Y, Guo X, Heng BC, Tong GQ. Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate. Reprod Biomed Online. 2010;20:510-5.

14. Machtinger R, Racowsky C. Morphological systems of human embryo assessment and clinical evidence. Symposium: Reprod Biomed. 2013;26:210-21.

15. Gardner DK, Sakkas D. Assessment of embryo viability: the ability to select a single embryo for transfer: a review. Placenta. 2003;24:5-12.

16. Alpha (Scientists in Reproductive Medicine) and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26:1270-83.

17. Van Den Bergh M, Ebner T, Elder K. Atlas of oocytes, Zygotes and Embryos in Reproductive Medicine Cambridge University Press; 2012.

18. Gardner DKG, Weissman A, Howles CM, Shohman Z, editors. Textbook of Assisted Reproductive Technologies, Laboratory and Clinical Perspectives. 3rd ed. London: Informa Health care; 2009.

19. Bączkowski T, Kurzaw R, Wojciech G. Methods of embryo scoring in in vitro fertilization. Reprod Biol. 2004;4(1):5-22.

20. Brezinova J, Oborna I, Svobodova M, Fingerova H. Evaluation of day one embryo quality and IVF outcome: a comparison of two scoring systems. Reprod Biol Endocrinol. 2009;7:9.

21. Gianaroli L, Magli MC, Ferraretti AP, Fortini D, Grieco N. Pronuclear morphology and chromosomal abnormalities as scoring criteria for embryo selection. Fertil Steril. 2003;80(2):341-9.

22. Fujimoto VY, Browne RW, Bloom MS, Sakkas D, Alikani M. Pathogenesis, developmental consequences, and clinical correlations of human embryo fragmentation. Fertil Steril. 2011;15:1197-204.

23. Johansson M, Hardarson T, Lundin K. There is a cutoff limit in diameter between a blastomere and a small anucleate fragment. J Assist Reprod Genet. 2003;20(8):309-13.

24. Magli CM, Jones GM, Lundin K, Etienne VA. Atlas of Human Embryology: from Oocytes to Preimplantation Embryos: Human Reproduction, Oxford University Press; 2012.

25. Desai N, Ploskonka S, Goodman LR, Austin C, Goldberg J, Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. Reprod Biol Endocrinol. 2014;12:54.

26. Hnida C, Agerholm I, Ziebe S. Traditional detection versus computer-controlled multilevel analysis of nuclear structures from donated human embryos. Hum Reprod. 2004;20(3): 665-71.

27. Pelinck MJ, De Vos M, Dekens M, Van der Elst J, De Sutter P, Dhont M. Embryos cultured *in vitro* with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod. 1998;13(4):960-3.

28. Sakkas D, Shoukir Y, Chardonnens D, Grace Bianchi P, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod. 1998;13:182-7.

29. Abe H, Otoi T, Tachikawa S, Yamashita S, Satoh T, Hoshi H. Fine structure of bovine morulae and blastocysts *in vivo* and *in vitro*. Anat Embryol. 1999;199(6):519-27.

30. Ahlstro A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. Hum Reprod. 2011;26(12):3289-96.

31. Gleicher N, Kushnir VA, Barad DH. Is it time for a paradigm shift in understanding embryo selection? Reprod Biol Endocrinol. 2015;13:3.

32. Veeck L, Zaninovic N. Human blastocysts in vitro. An Atlas of Human Blastocysts. Boca Raton, London, New York, Washington: Parthenon Publishing; 2009. p. 99-137.

33. Kovačić B, Vlaisavljević V. Importance of Blastocyst Morphology in Selection for Transfer. In: Wu DB, editor. Advances in Embryo Transfer. Rijeka, Hrvatska: In Tech; 2012. p. 161-77.

34. Kovačić B, Vlaisavljević V, Reljić M, Cizek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online. 2004;8:687-94.

35. Zhao Q, Yin T, Peng J, Zou Y, Yang J, Shen A, et al. Noninvasive metabolomic profiling of human embryo culture media using a simple spectroscopy adjunct to morphology for embryo assessment in *in vitro* fertilization (IVF). Int J Mol Sci. 2013;14:6556-70.

36. Katz-Jaffe MG, McReynolds S, Gardner DK, Schoolcraft WB. The role of proteomics in defining the human embryonic secretome. Mol Hum Reprod. 2009;15(5):271-7.

37. McReynolds S, Vanderlinden L, Stevens J, Hansen K, Schoolcraft WB, Katz-Jaffe MG. Lipocalin-1: a potential marker for noninvasive aneuploidy screening. Fertil Steril. 2011; 95(8):2631-3.

38. Wang SXY. The Past, present, and future of embryo selection in *in vitro* fertilization. Frontiers in reproduction conference. Yale J Biol Med. 2011;84(4):487-90.

39. Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril. 2012; 98(6):1481-e10.

40. Kirkegaard K, Hindkjaer JJ, Grondahl ML, Kesmodel US, Ingerslev HJ. A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. J Assist Reprod Genet. 2012;29:565-72.

41. Radulović S. Asistirana reproduktivna tehnologija u humanoj reprodukciji Beograd: Elit-Medica; 2007.

42. Harton G, Braude P, Lashwood A, Schmutzler A, Traeger-Synodinos J, Wilton L, et al. ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. Hum Reprod. 2010;26(1):14-24.

43. Zakon o lečenju neplodnosti postupcima biomedicinski potpomognutog oplođenja („Sl. glasnik RS“72/2009).

44. Kopitović V, Milatović S, Trninić-Pjević A, Bjelica A, Bujas I, Tabš N. Rezultati i iskustva nakon hiljadu ciklusa vantelesne oplodnje na Klinici za ginekologiju i akušerstvo u Novom Sadu. Med Pregl. 2011;64(11-12):565-9.

Rad je primljen 14. XII 2015.
Recenziran 20. XII 2015.
Prihvaćen za štampu 21. XII 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:241-246.

Clinical Center of Niš
Department of Gynecology and Obstetrics[1]
Faculty of Medicine, Niš[2]

Seminar for physicians
Seminar za lekare u praksi
UDK 618.173-085:615.357
DOI: 10.2298/MPNS1608247K

MENOPAUSAL HORMONE THERAPY: BENEFITS AND DIFFERENT FORMS

HORMONSKA TERAPIJA U MENOPAUZI: KORISNI EFEKTI I RAZLIČITI OBLICI

Ranko M. KUTLEŠIĆ[1,2], Jasmina POPOVIĆ[1,2], Milan STEFANOVIĆ[1,2], Predrag VUKOMANOVIĆ[1,2], Aleksandra ANDRIĆ[1] and Jelena MILOŠEVIĆ[1,2]

Summary

Introduction. Declining of ovarian hormone production can seriously disturb the quality of woman's life, with physical and emotional consequences and to potentiate the development of additional health risks such as cardiovascular diseases and osteoporosis which are already present in women of older age. **Benefits of Menopausal Hormone Therapy.** Menopausal hormone therapy ameliorates the quality of life by resolving the atrophic symptoms and vasomotor problems, protecting from the osteoporosis, maintaining the skin and connective tissue turgor, as well as by improving libido, mood and depression during the menopausal transition. **Forms of Menopausal Hormone Therapy.** There are several possibilities to treat menopausal problems: estrogen, combination of estrogen and progestogen, androgens, selective estrogen receptor modulators, tissue selective estrogen complex, tibolon and alternatives. **Initiating, Monitoring and Discontinuing Menopausal Hormone Therapy.** Menopausal hormone therapy should be started when the problems due to menopausal symptoms appear. It is important to have on mind that the effects of hormones depend on age and actual condition of the woman's organism. The goal is effective treatment at the lowest dose and during the shortest interval needed for symptom control. The therapy must be reevaluated every year and potential risks must be discussed as well. **Conclusion.** Menopausal hormone therapy ameliorates the quality of woman's life in perimenopause. Type, doses and duration of the menopausal hormone therapy should be individualized.

Key words: Hormone Replacement Therapy; Menopause; Treatment Outcome; Quality of Life; Symptoms and Signs; Risk Factors; Bone Density; Estrogen Replacement Therapy; Progestins; Androgens; Drug Compounding

Sažetak

Uvod. Pad produkcije hormona jajnika može bitno da naruši kvalitet života žene, da ima fiziološke i emocionalne posledice i potencira razvoj dodatnih rizika po zdravlje koji ionako postoje u starijem životnom dobu, kao što su to kardiovaskularni poremećaji i osteoporoza. **Korisni efekti hormonske terapije u menopauzi.** Hormonska terapija u menopauzi rešava simptome atrofije i vazomotorne probleme; štiti od osteoporoze; održava turgor kože i vezivnog tkiva; popravlja libido, raspoloženje i depresivne simptome tokom perimenopauze. **Forme hormonske terapije u menopauzi.** Mogućnosti za tretman problema u menopauzi su: estrogen, kombinacija estrogena i progestagena, androgeni, selektivni modulatori estrogenih receptora, tkivnoselektivni estrogeni kompleks, tibolon i alternative. **Kada započeti hormonsku terapiju, monitoring tokom primene i kada je obustaviti?** Hormonsku terapiju u menopauzi treba započeti kada se jave problemi zbog simptoma menopauze i pritom treba imati u vidu da efekti hormona na organizam žene zavise od životnog doba i od trenutnog stanja samog organizma. Cilj je efikasan tretman uz što manju dozu i trajanje, reevaluacija svake godine, uz razmatranje mogućeg rizika. **Zaključak.** Hormonska terapija u menopauzi poboljšava kvalitet života žene u perimenopauzi. Tip, doza i trajanje hormonske terapije u menopauzi zavisi od ciljeva tretmana i konkretnih rizika i treba da se odrede za svaku pacijentkinju ponaosob.

Ključne reči: supstituciona hormonska terapija; menopauza; ishod lečenja; kvalitet života; simptomi i znaci; faktori rizika; gustina kostiju; estrogenska supstituciona terapija; progestini; androgeni; kombinovanje lekova

Introduction

Menopause is not a pathological, but quite a normal life event. Still, declining hormone production can essentially disturb the quality of woman's life, with physical and emotional consequences, and, which is more important, it may potentiate the development of additional health risks, such as cardiovascular diseases and osteoporosis already present in women of older age.

The aim of this article is to present current treatment of perimenopausal problems.

Benefits of Menopausal Hormone Therapy

The benefits of menopausal hormone therapy (MHT) are obvious - it efficiently solves the menopausal problems, such as vasomotor symptoms (hot flushes), atrophic changes (superficial dyspareunia and vaginal dryness), urinary problems (frequent urination and urgency), etc. [1–6].

Corresponding Author: Doc. dr Ranko M. Kutlešić, Klinika za ginekologiju i akušerstvo, 18000 Niš, Bulevar Zorana Đinđića 48, E-mail: kutlesicr@medianis.net

Abbreviations

MHT	– menopausal hormone therapy
CRP	– C reactive protein
SHBG	– sex hormone binding globuline
Es	– estrogen
Pr	– progesterone
HDL	– high density lipoprotein
SERM	– selective estrogen receptor modulators
MORE	– Multiple Outcome of Raloxifene Evaluation study
STAR	– Study of Tamoxifen and Raloxifene
RUTH	– Raloxifene Use for The Heart study
TSEC	– Tissue-Selective Estrogen Complex
LDL	– low density lipoprotein
TREATS study	– The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening study
VTE	– venous thromboembolism

The most important systemic effect of MHT is preserving the bone density, preventing osteoporosis and diminishing the incidence of intravertebral and hip fractures. Estrogen replacement is a therapeutic measure for prevention and treatment of osteoporosis in women with premature ovarian failure and women under 60 years of age. And yet, it is not recommended to begin or continue MHT after the age of 60 only for prevention of osteoporosis [2]. Studies have revealed that the protective effect on bone tissue could be achieved even at lower doses than recommended 2 mg/day of oral estradiol or 0.05 mg/day of transdermal estradiol or 0.625 mg/day of conjugated equine estrogen [7].

Estrogen replacement has a favorable effect on metabolism not only on the level of bone matrix, but also on the skin, intervertebral disks and arterial tunica media, protecting them from the loss of connective tissue [8].

The level of bone protection declines after discontinuation of MHT. However, some studies have shown that the use of MHT several years after the menopause could have long-term protective effects on bone [9].

Favorable systemic effects also include the improvement of libido, mood and depressive symptoms during menopausal transition and early postmenopause. Nevertheless, MHT is not an alternative to antidepressants when indicated for chronic depression [10].

During early postmenopausal period the short-term use of MHT may improve cognitive functions and it might prevent Alzheimer's disease, but more adequate investigations are needed for definitive proofs [1]. Large studies have revealed that MHT does not improve memory or cognitive function in older postmenopausal women, even if there was an association of MHT and increased risk of dementia in older women (between the ages of 65 and 79 years). [1] Based on actual recommendations, MHT should not be started during postmenopausal period just for the improvement of cognitive functions or for decreasing the risk from dementia [1, 11].

Menopausal hormone therapy (MHT) is recommended not only to control vasomotor problems and atrophic changes in the patients with premature ovarian failure, but also to slowdown atherosclerosis, to decrease the risk from cardiovascular diseases, osteoporosis and, possibly, Alzheimer's disease [11, 12].

Most women tolerate MHT well, but side effects of standard doses are possible, such as breast tenderness, vaginal bleeding, the sense of swelling (due to water retention) and headaches [6]. The use of MHT could be also associated with depression and mood changes.

Forms of Menopausal Hormone Therapy

Different treatment options for menopausal symptoms are listed in **Table 1.**

Estrogen

The use of estrogen is the oldest way to treat menopausal problems. Estrogen is successful in solving vasomotor symptoms, atrophic changes and their consequences (dyspareunia, vaginal dryness and urinary problems). Daily doses of estrogen are shown in **Table 2**.

There are two routs of estrogen administration: oral and transdermal (using patches or subcutaneous implants).

Orally administered estrogen follows the effect of first liver pass, which could disturb coagulation cascade (hypercoagulability as an end effect) and could also make changes in markers of inflammation including C-reactive protein [13]. Oral estradiol is converted into estrone in the liver and intestinum, which is not a case with transdermal estradiol. Oral estrogens increase hepatic synthesis of sex-hormone binding globulin (SHBG), decreasing the levels of free testosterone, which could result in lower libido [14].

Local (vaginal) estrogen administration is approved when there are only atrophic changes. Locally administered estrogen regenerates the atrophic vaginal epithelium and resolves dyspareunia successfully. The treatment effect could be monitored by measuring the vaginal pH – it should be less than 4.5. Although local absorption is small, it does exist; it is even increased through atrophic vaginal mucosa [15]. This is clinically important: in cases of topical estrogen treatment longer than 6 months, the thickness of the endometrium should be measured by transvaginal ultrasound and biopsy should be performed if indicated. Bearing in mind estrogen effects on breast tissue, breast control is also mandatory during the treatment. The current recommendation is to use the lowest estrogen dose which could control symptoms since the safety of using vaginal estrogen preparations for the period longer than one year has not been proved [1]. Transdermal or local use of estrogen is more adequate for patients over 60 years of age [11].

Combination of Estrogen and Progestogen

An endometrial response to unopposed estrogen is hyperplasia, so progestogen is added to protect the endometrium against the uncontrolled proliferation (progestogen induces endometrial secretory transformation). There are two ways to administer the combination of estrogen and progestogen: the sequential and continuous regimen.

Table 1. Treatment of menopausal symptoms
Tabela 1. *Tretman simptoma u menopauzi*

- estrogen/*estrogen*
- combination of estrogen and progestogen/*kombinacija estrogena i progestagena*
  - sequential regimen/*sekvencijalni režim*
  - continuous regimen/*kontinuirani režim*
- androgens/*androgeni*
- SERM – selective estrogen receptor modulator/*selektivni modulatori estrogenih receptora*:
  - raloxifene/*raloksifen*
  - ospemifene/*ospemifen*
- TSEC - tissue-selective estrogen complex/*tkivno selektivni estrogeni kopleks*
- tibolone/*tibolon*
- alternative treatments/*alternativne terapije i postupci*

The sequential regimen means that estrogen (Es) is administered every day and progestogen (Pr) is used for 14 days (minimum 10 days). The patient has cyclic progestin withdrawal bleeding and this regimen is suitable for patients in the beginning of perimenopause.

The continuous regimen means the administration of Es+Pr combination every day, and there is no withdrawal bleeding. This regimen is more suitable for postmenopausal patient who does not want to have cyclic bleedings.

Daily doses used in Es+Pr combination are shown in **Table 2**. It is better to add progestogen in the evening; metabolites pregnenolon and alopregnenolon improve sleeping [15].

Micronized oral progesterone seems to be a better option in comparison with synthetic progestogens because it has no metabolites with androgenic and glucocorticoid activities. On the contrary, it has slightly hypotensive effects due to its anti-mineralocorticoid performances as well as the overall favorable cardiovascular impact (decreasing the risk of venous thromboembolia, probably the stroke). Micronized oral progesterone has lower mitogenic activity on the breast tissue than medroxyprogesteron acetate in synergism with estrogen [16].

There are alternatives to oral Es+Pr combination, such as adding of progesterone in the form of vaginal gel [17], combination of transdermal estradiol and micronized oral progesterone [18] and combination of oral estrogen and intrauterine device with progestogen – levonorgestrel [19]. Intrauterine administration of progestogen, especially levonorgestrel, as a part of MHT, is considered safe and efficient in the endometrial protection [19]. This is also true for progesterone administered in the form of vaginal gel or pessaries. [1] However, the effects of progestogen administered in such a way on the bone and breast as well as other systemic effects have not been completely tested.

The use of combined (Es+Pr) MHT could cause the occurrence of progestogenic side effects, including breast tenderness, swelling due to body water retention, headaches, mood changes and depression. Progestogen administration in the forms of vaginal gel, pessaries or levonorgestrel releasing intrauterine system reduces systemic side effects; however, they could not be completely eliminated. Continuous combined regimens are associated with continuous low-grade side effects of progestogen [1].

Complications of sequential combined regimen in form of irregular bleedings do not practically exist, so every irregular bleeding must arouse the suspicion of possible organic causes and require the prompt evaluation.

A relatively frequent complication of continuous (Es+Pr) combined regimen is breakthrough bleeding, with the same pathogenesis with oral contraceptive use, usually disappearing after 6 – 12 months of administration. If the breakthrough bleedings are persistent, it is possible to start with the sequential regimen or to apply intrauterine device with progestogen. Alternatives are endometrial ablation or even vaginal hysterectomy [15]. The main question is when to perform endometrial biopsy. Endometrial biopsy is needed before the treatment with Es+Pr combination in the patients at risk factors for endometrial proliferation or in patients who have used unopposed estrogen as MHT at any time. In case of the solicitude of the patient or her gynecologist during the treatment, biopsy is justifiable, which is mandatory in cases of irregular bleeding during the treatment with unopposed estrogen, persistence of bleeding after 6 months of continuous combined (Es+Pr) therapy or if endometrial thickness is more than 4 mm on transvaginal ultrasound examination in a postmenopausal patient. Standard combined regimens (Es every day + Pr 14 days or Es+Pr every day) protect the endometrium, but every change (lower dose or shorter duration) requires careful monitoring of endometrium [15].

Combined (Es + Pr) systemic therapy is associated with an increased risk of venous thromboembolism, ischemic stroke and proliferation of breast tissue. The latter risk decreases after the treatment has been discontinued [4].

Androgens

Androgens are added as short-term MHT because of their favorable psychological effects and to improve the libido, but only high doses have such effects. Methyltestosterone (synthetic androgen) could be used at a dose of 1.25 to 2.5 mg a day [15]. Androgens could be administered orally, intramuscularly or in

Table 2. Estrogen and progestogen daily dose in MHT
Tabela 2. *Dnevne doze estrogena i progestagena koje se koriste u hormonskoj terapiji u menopauzi*

Estrogen dose/*Doze estrogena*	
Standard dose/*Standardna doza*	Low dose/*Niska doza*
conjugated equine estrogen 0.625 mg *konjugovani ekvini estrogen 0,625 mg* micronized estradiol 1 – 2 mg *mikronizovani estradiol 1–2 mg* ethinyl estradiol 5 μg/*etinil estradiol 5 μg* estradiol valerate 2 mg/*estradiol valerat 2 mg*	conjugated estrogen 0.3 – 0.45 mg *konjugovani estrogen 0,3–0,45 mg* micronized estradiol 0.5 mg *mikronizovani estradiol 0,5 mg* ethinyl estradiol 2.5 μg *etinil estradiol 2,5 μg*
Progestogen dose/*Doze progestagena*	
Standard dose/*Standardna doza* (sequential regimen/*sekvencijalni režim*)	Low dose/*Niska doza* (continuated regimen/*kontinuirani režim*)
medroxyprogesterone acetate 5 mg *medroksiprogesteron acetat 5 mg* norethindrone 0.7 mg/*noretindron 0,7 mg* norethindrone acetate 1 mg/*noretindron acetat 1 mg* micronized progesterone 200 mg *mikronizovani progesteron 200 mg*	medroxyprogesterone acetate 1.5 – 2.5 mg *medroksiprogesteron acetat 1,5–2,5 mg* norethindrone 0.35 mg/*noretindron 0,35 mg* norethindrone acetate 0.5 or 1 mg *noretindron acetat 0.5 mg ili 1 mg* micronized progesterone 100 mg *mikronizovani progesteron 100 mg*

the form of gel, patches or implants [1]. On the other side, androgens have no influence on hot flushes and breakthrough bleedings (they do not protect the endometrium) [15], and density of the bones can be improved only by their high doses or in combination with estrogen. Other treatment options include transdermal testosterone administration or dehydroepiandrosterone treatment [20].

Unwanted effects of androgen use are hirsutism, acne and alopecia, as well as the unfavorable effect on the lipid profile, i.e. decreasing the levels of high density lipoproteins (HDL), the latter one is lost with parenteral administration. Most guidelines do not recommend androgens for hormone replacement in women [21], and if used, it is mandatory to monitor the patient's lipid status and the occurrence of symptoms and signs of androgen excess along with measuring the serum testosterone, which should be in physiological levels between 0.7 and 2.8 nmol/l for women [15].

Selective Modulators of Estrogen Receptors

New agents for the control of menopausal symptoms are introduced in order to avoid steroid action on the endometrial and breast tissue proliferation: selective estrogen agonists/antagonists or selective estrogen receptor modulators (SERM). The second generation of SERM has no proliferative action on the endometrium as was the case with previously used tamoxifen. The second SERM generation includes raloxifene, and newer SERM molecules are bazedoxifene, lasofixene, teremifene, ospemifene and arzoxifene [22, 23]. Raloxifene administered at an oral dose of 60 mg a day is the one most frequently used.

Raloxifen is the only SERM internationally approved for prevention and treatment of osteoporosis and vertebral fractures. Raloxifene improves the bone density and lipid profile. Extensive MORE (Multiple Outcome of Raloxifene Evaluation) study has revealed that raloxifene reduces the risk of vertebral fractures, acts as antiestrogen on the breast tissue (according to STAR – Study of Tamoxifen and Raloxifene), but has no significant action on vasomotor symptoms and atrophic changes [24, 25]. RUTH (Raloxifene Use for The Heart) study has shown that raloxifene does not affect cardiovascular diseases [26]. Raloxifene should be used in the patients who need the protection from osteoporosis, but who do not want or should not take hormone replacement therapy containing steroids.

Ospemifene at an oral dose of 60 mg/day (30 – 90 mg/day) is most effective in the control of atrophic changes, it successfully reduces moderate and severe dyspareunia. Side effects of ospemifene are hot flushes, excessive sweating, increased vaginal secretion and muscular spasms. Ospemifene does not influence the endometrial thickness [6, 27].

The main concerns with SERMs are thrombotic events: raloxifene increases the risk from thrombosis [26], but this is not a case with ospemifene [27].

Selective estrogen receptor modulators (SERMs) are a good treatment option for younger patients at an increased risk for fractures, who have to take the long-term therapy, and for the patients without a risk for thrombosis, but who have contraindications for other treatments [28]. Nowadays, studies are being performed to find an "ideal" SERM which would have the estrogen-like effect on the bones and lipids while being neutral on the level of endometrium and having antiestrogenic action on the breast, without side effects, especially on coagulation profile [22].

Tissue-Selective Estrogen Complex

Tissue-selective estrogen complex (TSEC) is a concept developed to control vasomotor and atrophic symptoms, to maintain the bone density and to avoid proliferative effects on the endometrium and

breast. TSEC is a combination of one or more estrogens and selective estrogen receptor modulator [29]. In practice this is the combination of conjugated estrogen (at a daily dose of 0.625 mg or 0.3 mg or 0.45 mg) and bazedoxifene (20 mg/day) [30]. Bazedoxifene is chosen because it can inhibit the action of conjugated estrogen on the endometrium [31]. Clinical studies have revealed that the combination of conjugated estrogen (0.45 mg or 0.625 mg) and bazedoxifene (20 mg) is a good alternative to the traditional Es+Pr MHT in the control of vasomotor symptoms, vulvovaginal atrophy and dyspareunia, as well as in the prevention of osteoporosis in postmenopausal women with uterus [32].

With TSEC as MHT, the use of progestogen and its side effects are also avoided, thus contributing to further individualization of therapy for menopausal symptoms [33]. Additional studies are needed to determine the long-term safety of TSEC on the cardiovascular system and breast [34].

Tibolon

Tibolon molecule is structurally similar to 19-nortestosterones, binding to the estrogen receptor. Its 3-OH metabolites have 100 times higher affinity for α-estrogen receptor (ER) comparing to β-ER, and Δ4-ketoisomer metabolite has androgenic and progestogenic effects. It is administered at a daily dose of 2.5 mg (1.5 mg). Clinical studies have revealed that tibolon successfully improves the bone mineral density and symptoms of urogenital atrophy [35]. The impact of tibolone on the libido is very impressive (androgen metabolites), especially on mood, which we have also seen in our clinical practice.

Tibolon can stimulate the endometrium very rarely: tibolon metabolite Δ4-ketoisomer, which is dominant on the level of endometrium, binds to progesterone receptors and protects the endometrium from metabolites with estrogenic activity. Nevertheless, close monitoring of endometrial thickness is needed in patients on tibolone and biopsy is mandatory in cases with uterine bleeding [15].

In the beginning of tibolon use, it was considered that tibolon could absolutely protect the breast tissue from proliferation, due to inhibitory action of its metabolites on sulphatase, the consequence is inhibited conversion of estrone sulfate to estradiol. Nevertheless, tibolone could increase the breast tissue density on mammography [35]. In fact, tibolon acts as an androgen and progestogen on the breast. Regular control of the breast is mandatory on patient on tibolone, as during any MHT.

Due to its androgenic effects, tibolone influences the lipid profile, decreasing the level of HDL, but it has a neutral effect on low density lipoproteins (LDL) [36].

Bioidentical Hormone Therapy

Menopausal hormone therapy is available not only in the form of commercial preparations, but there is also a possibility to compose components depending on individual needs of the patient. "Bioidentical hormone" preparations are often used to such purpose. It has become very popular in the United States due to the fear resulting from the findings of Women Health Initiative (WHI) study and a widespread opinion that the natural therapy is the best therapy [37]. The term "bioidentical hormone therapy" is a commercial term, and it is not based on scientific evidence. "Bioidentical hormone therapy" means the use of bioidentical hormones, derived from plants (e.g. soya) and chemically modified to be similar or structurally identical to human endogenous hormones. Different doses and routs of administration are used. These preparations could be efficient in the control of menopausal symptoms [38, 39].

The issue of their safety as compared with traditional forms of hormones is very important. Theoretically, the risk should be the same as for conventional forms of MHT, but there is still the question of their bioavailability, problems with sub- or overdosing and effect of different combinations. There are no adequate studies about pharmacokynetics, effectiveness and possible risks from these preparations. The current opinion is that their use is less favorable than of conventional MHT [40]. Moreover, there are guidelines that do not recommend the use of custom-compounded bioidentical hormone therapy [4], and others that allow the use of that therapy only in cases of allergies to ingredients contained in conventional MHT [5].

Introduction, Monitoring and Discontinuation of MHT

Menopausal hormone therapy should be initiated when menopausal problems appear. MHT is not obligatory for every woman, but it would be ideal if every woman were informed about benefits and risks associated with MHT. It must be kept in mind that the effects of hormones are determined by the age and current health condition, so the concepts of "window of opportunity" and "timing hypothesis" have been developed [11, 41]. It is generally accepted that the benefits from MHT overcome the risks for symptomatic women who are under 60 years of age or within 10 years of menopause, especially if other, non-hormonal treatments have had no effects. In older women and women having been in postmenopause for more than ten years, the risk/benefit ratio is less favorable [4, 11]. Current recommendations for patients with premature ovarian failure are that MHT should be used at least to the age when natural menopause occurs [1–6, 42, 43].

The preventive effect of MHT on the development of osteoporosis is the only one proved up to now [44], but it is not always the case with the prevention of cardiovascular diseases [45]. MHT should not be used either for primary or secondary prevention of stroke [1] or dementia [12]. To be more specific, the standard-dose of estrogen-alone MHT may decrease the progression of atherosclerosis and incidence of coronary heart disease (and all-cause mortality as well) in wo-

men younger than 60 years of age or within 10 years of menopause [4]. The combination of estrogen and progestogen MHT in this population has no significant effects on coronary heart disease [4]. If MHT is initiated at an older age, it could destabilize atherosclerotic plaques and increase the risk from myocardial infarction and stroke [41].

When estimating the risk for thromboembolic complication associated with MHT, it is necessary to take into account the presence of the individual risk factors for every patient [46]. TREATS study (The **T**hrombosis: **R**isk and **E**conomic **A**ssessment of **T**hrombophilia **S**creening) has shown that genetic affinity to thrombophilia has also an important role in the occurrence of increased risk for venous thromboembolism (VTE) in MHT users who are carriers of factor V Leiden mutation [47].

The choice of route, form and dose of MHT depends on the age and health status of the individual patient, time interval from menopause, the treatment goals and presence of specified contraindications, along with identification of individual risk factors (taken from family medical history as well). The target is to control the symptoms and achieve the wanted effect at the lowest possible dose and in the shortest time interval [5, 11]. Introduction of MHT in women using transdermal estradiol in combination with micronized progesterone in their early and mid 50s is now believed to be associated with a lower risk [2].

Monitoring During Administration of MHT

The first control should be taken after few months of using MHT to establish the effects and adjust the dose, if necessary. Reevaluation is mandatory after one year, with consideration of possible risks. It is recommended to take once a year the clinical breast examination (and mammography for older than 40) as well as pelvic examination, to assess the symptom control and possible occurrence of new health risks, to discuss the possibilities of new or alternative treatments, as well as to evaluate woman's individual needs [11].

Discontinuing MHT

It is generally recommended to limit MHT use to the shortest interval and lowest dose needed to achieve treatment goals. Duration of the MHT should be limited to 3 to 5 years in women having reached menopause around the average age due to breast risks [5, 11, 48]. Studies have shown that estrogen only therapy has no such risk even after 7 years of use [5, 11, 49]. For some patients, e.g. those at high risk from osteoporotic fractures, in whom alternative treatments are not appropriate or tolerated, MHT use could be extended for longer intervals if the patients are provided with the information about benefits and risks and undergo appropriate clinical supervision [5, 11].

After MHT has been discontinued, recurrence of vasomotor symptoms is possible in 50%; independent of age, duration of use and the way of discontinuing MHT (gradually or not) [5, 11].

In everyday clinical practice with patients requiring MHT, numerous guidelines could be very helpful, but the most useful is the advice of L. Speroff: ''let the patient be your guide" [15].

Conclusion

Menopause is not a pathological, but normal life event. Hormone therapy is not the standard for every perimenopausal women, but it would be ideal if every woman could be informed on the benefits and risks of menopausal hormone therapy.

Menopausal hormone therapy improves the quality of life in perimenopause by solving the symptoms of urogenital atrophy and controlling vasomotor problems, protecting from osteoporosis, maintaining the skin and connective tissue turgor and improving the libido and mood.

Every individual patient having menopausal problems should have the therapy determined and individual risk/benefit ratio estimated. The treatment goal is the effective treatment at the lowest dose and the shortest duration of the treatment possible.

The decision about the form, dose and duration of menopausal hormone therapy should be individual for every patient. It should be reevaluated once a year, with risk reassessment. It must be taken into account that the effects of hormones on woman's organism depend on age and the current health status. It is generally accepted that the benefit from menopausal hormone therapy surpass the risk in patients under 60 years of age or if started within 10 years of natural menopause, especially if other, non-hormonal treatments have failed. In women older than 60 or after 10 years from menopause, lower doses are needed (transdermal or locally), and risk/benefit ratio from menopausal hormone therapy is less favorable. Patients with premature ovarian failure should use menopausal hormone therapy at least until the age of average menopause.

References

1. Panay N, Hamoda H, Arya R, Savvas M. The 2013 British Menopause Society & Women's Health Concern recommendations on hormone replacement therapy. Menopause International 2013;19(2):59-68.

2. Hamoda H, Savvas M. What should we be advising women about hormone replacement therapy? InnovAiT: Education and inspiration for general practice 2014;7(5):95-300.

3. de Villiers TJ, Pines A, Panay N, Gambacciani M, Archer DF, Baber RJ, et al. International Menopause Society. Updated 2013 International Menopause Society recommendations on menopausal hormone therapy and preventive strategies for midlife health. Climacteric. 2013;16(3):316-37.

4. de Villiers TJ, Gass ML, Haines CJ, Hall JE, Lobo RA, Pierroz DD, et al. Global consensus statement on menopausal hormone therapy. Climacteric. 2013;16(2):203-4.

5. Schmidt P. The 2012 Hormone Therapy Position Statement of The North American Menopause Society. Menopause. 2012;19(3):257-71.

6. ACOG Practice Bulletin No. 141: management of menopausal symptoms. Obstet Gynecol. 2014;123(1):202-16.

7. Prestwood KM, Kenny AM, Kleppinger A, Kulldorff, M. Ultra low dose micronized 17beta-estradiol and bone density and bone metabolism in older women: a randomized controlled trial. J Am Medical Association. 2003;290(8):1042-8.

8. Cirillo DJ, Wallace RB, Wu L, Yood RA. Effect of hormone therapy on risk of hip and knee joint replacement in the Women's Health Initiative. Arthritis & Rheumatism. 2006;54:3194-204.

9. Middleton ET, Steel SA. The effects of short-term hormone replacement therapy on long-term bone mineral density. Climacteric. 2007;10(3):257-63.

10. Bromberger JT, Schott LL, Kravitz HM,Sowers M, Avis N E, Gold EB, Matthews KA. Longitudinal change in reproductive hormones and depressive symptoms across the menopausal transition: Results from the Study of Women's Health Across the Nation (SWAN). Archives of General Psychiatry. 2010;67:598-607.

11. Sood R, Faubion SS, Kuhle CL, Thielen JM, Shuster LT. Prescribing menopausal hormone therapy: an evidence-based approach. Int J Womens Health. 2014;6:47-57.

12. Maki PM, Henderson VW. Hormone therapy, dementia, and cognition: the Women's Health Initiative 10 years on. Climacteric. 2012;15(3):256-62.

13. Goodman MP. Are all estrogens created equal? A review of oral vs transdermal therapy. J Womens Health (Larchmt). 2012;21(2):161-9.

14. Arlt W. Androgen therapy in women. Eur J Endocrinol 2006;154(1):1-11.

15. Sperof L. Postmenopausal hormone therapy. In Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. 7th ed. Philadelphia: Lippincot Williams&Wilkins; 2005. p. 689-777.

16. L'Hermite M. HRT optimization, using transdermal estradiol plus micronized progesterone, a safer HRT. Climacteric. 2013;16 Suppl 1:44-53.

17. Almomen A, Cho S, Yang CH, Li Z, Jarboe EA, Peterson CM, Huh KM, Janát-Amsbury MM. Thermosensitive progesterone hydrogel: a safe and effective new formulation for vaginal application. Pharm Res. 2015;32(7):2266-79.

18. Simon JA. What's new in hormone replacement therapy: focus on transdermal estradiol and micronized progesterone. Climacteric. 2012;15 Suppl 1:3-10.

19. Riphagen FE Intrauterine application of progestins in hormone replacement therapy: a review. Climacteric. 2000;3(3):199-211.

20. Arlt W. Androgen therapy in women. Eur J Endocrinol. 2006;154(1):1-11.

21. Wierman ME, Arlt W, Basson R, Davis SR, Miller KK, Murad MH, et al. Androgen therapy in women: a reappraisal: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(10):3489-510.

22. Maximov PY, Lee TM, Jordan VC. The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice. Curr Clin Pharmacol. 2013;8(2):135-55.

23. Ellis AJ, Hendrick VM, Williams R, Komm BS. Selective estrogen receptor modulators in clinical practice: a safety overview. Expert Opin Drug Saf. 2015;14(6):921-34.

24. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA. 1999;282(7):637-45.

25. Vogel VG.The NSABP Study of Tamoxifen and Raloxifene (STAR) trial. Expert Rev Anticancer Ther. 2009;9(1):51-60.

26. Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, et al. Raloxifene use for the heart (RUTH) trial investigators. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355(2):125-37.

27. Goldstein SR, Archer DF, Simon JA, Constantine G. Endometrial safety of ospemifene and the ability of transvaginal ultrasonography to detect small changes in endometrial thickness. Obstet Gynecol. 2014;123 Suppl 1:96S-7S.

28. Komm BS, Mirkin S. An overview of current and emerging SERMs. J Steroid Biochem Mol Biol. 2014;143:207-22.

29. Mirkin S, Komm BS. Tissue-selective estrogen complexes for postmenopausal women. Maturitas. 2013;76(3):213-20.

30. Kagan R. The tissue selective estrogen complex: a novel approach to the treatment of menopausal symptoms. J Womens Health (Larchmt). 2012;21(9):975-81.

31. Mirkin S, Archer DF, Taylor HS, Pickar JH, Komm BS. Differential effects of menopausal therapies on the endometrium. Menopause. 2014;21(8):899-908.

32. Komm BS, Mirkin S, Jenkins SN. Development of conjugated estrogens/bazedoxifene, the first tissue selective estrogen complex (TSEC) for management of menopausal hot flashes and postmenopausal bone loss. Steroids. 2014;90:71-81.

33. Santen RJ, Kagan R, Altomare CJ, Komm B, Mirkin S, Taylor HS. Current and evolving approaches to individualizing estrogen receptor-based therapy for menopausal women. J Clin Endocrinol Metab. 2014;99(3):733-47.

34. Levine JP. Treating menopausal symptoms with a tissue-selective estrogen complex. Gend Med. 2011;8(2):57-68.

35. Bundred NJ, Kenemans P, Yip CH, Beckmann MW, Foidart JM, Sismondi P, et al. Tibolone increases bone mineral density but also relapse in breast cancer survivors: LIBERATE trial bone substudy. Breast Cancer Research. 2012;14:R13

36. Jeremić K, Lazović G, Tulić I, Gojnić M, Stojnić J, Jeremić J. Effects of tibolone on the risk factors for cardiovascular diseases in postmenopausal women. Vojnosanit Pregl. 2006;63(4):387-91.

37. Pfeifer S, Goldberg J, Lobo R, McClure RD, Thomas M, Widra E, et al. American college of obstetricians and gynecologists Cometee on gynecologic practice; American society for reproductive medicine Practice cometee. Compounded bioidentical menopausal hormone therapy. Fertil Steril. 2012;98(2):308-12.

38. Mirkin S, Amadio JM, Bernick BA, Pickar JH, Archer DF. 17β-Estradiol and natural progesterone for menopausal hormone therapy: REPLENISH phase 3 study design of a combination capsule and evidence review. Maturitas 2015;81(1):28-35.

39. Ruiz AD, Daniels KR. The effectiveness of sublingual and topical compounded bioidentical hormone replacement therapy in postmenopausal women: an observational cohort study. Int J Pharm Compd. 2014;18(1):70-7.

40. Pinkerton JV. What are the concerns about custom-compounded "bioidentical" hormone therapy? Menopause. 2014;21(12):1298-300.

41. Clarkson TB, Melendez GC, Appt SE. Timing hypothesis for post-menopausal hormone therapy: its origin, current status, and future. Menopause. 2013;20(3):342-53.

42. Ivanisević M, Vujović S, Drezgić M. Effects of estroprogestagens on lipid and hormonal profiles in women with premature primary ovarian failure. Med Pregl. 2005;58(5-6):296-300.

43. Vujović S, Ivović M, Tancić-Gajić M, Marina L, Barać M, Arizanović Z, et al. Premature ovarian failure. Srp Arh Celok Lek. 2012;140(11-12):806-11.

44. Vujasinović-Stupar N, Radojcić L, Nenadić D. Prevention of osteoporosis. Vojnosanit Pregl. 2007;64(3):205-10.

45. Bosković SD, Nesković AN. Atherosclerosis plaque regression. Med Pregl. 2006;59(1-2):38-45.

46. Tasić L, Dunjić R, Vasiljević M, Dikić S, Jurisić A, Tasić D. Comparison of two different types of hormone therapy effects on haemostatic parameters in early postmenopausal women. Srp Arh Celok Lek. 2011;139(1-2):52-7.

Rad je primljen 2. VIII 2015.
Recenziran 28. IX 2015.
Prihvaćen za štampu 8. X 2015.
BIBLID.0025-8105:(2016):LXIX:7-8:247-254.

47. Wu O, Robertson L, Langhorne P, Twaddle S, Lowe GD, Clark P, et al. Oral contraceptives, hormone replacement therapy, thrombophilias and risk of venous thromboembolism: a systematic review. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Thromb Haemost. 2005;94(1):17-25.

48. Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, et al, for the WHI Investigators. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal womenthe Women's Health Initiative Randomized Trial. JAMA. 2003;289(24):3243-53.

49. Stefanick ML, Anderson GL, Margolis KL, Hendrix, Rodabough RJ, Paskett ED, et al, for the WHI Investigators. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA. 2006;295(14):1647-57.

General Practice Health Center Zaječar, Serbia[1]
High Medical School of Professional Studies Ćuprija, Serbia[2]

Seminar for physicians
Seminar za lekare u praksi
UDK 614.2:614.8.028.4
DOI: 10.2298/MPNS1608255S

EMERGENCY RESPONSE TEAMS TRAINING IN PUBLIC HEALTH CRISIS – THE SERIOUSNESS OF SERIOUS GAMES

OBUKA TIMOVA ZA REAGOVANJE U JAVNOZDRAVSTVENIM KRIZAMA − OZBILJNOST OZBILJNIH IGARA

Vojislav STANOJEVIĆ[1] and Čedomirka STANOJEVIĆ[2]

Summary

Introduction. The rapid development of multimedia technologies in the last twenty years has lead to the emergence of new ways of learning academic and professional skills, which implies the application of multimedia technology in the form of a software –"serious computer games". **Three-Dimensional Virtual Worlds.** The basis of this game-platform is made of the platform of three-dimensional virtual worlds that can be described as communication systems in which participants share the same three-dimensional virtual space within which they can move, manipulate objects and communicate through their graphical representatives- avatars. **Medical Education and Training.** Arguments in favor of these computer tools in the learning process are accessibility, repeatability, low cost, the use of attractive graphics and a high degree of adaptation to the user. Specifically designed avatars allow students to get adapted to their roles in certain situations, especially to those which are considered rare, dangerous or unethical in real life. **Discussion**. Drilling of major incidents, which includes the need to create environments for training, cannot be done in the real world due to high costs and necessity to utilize the extensive resources. In addition, it is impossible to engage all the necessary health personnel at the same time. New technologies intended for conducting training, which are also called "virtual worlds", make the following possible: training at all times depending on user's commitments; simultaneous simulations on multiple levels, in several areas, in different circumstances, including dozens of unique victims; repeated scenarios and learning from mistakes; rapid feedback and the development of non-technical skills which are critical for reducing errors in dynamic, high-risk environments. **Conclusion**. Virtual worlds, which should be the subject of further research and improvements, in the field of hospital emergency response training for mass casualty incidents, certainly have a promising future.

Key words: Mass Casualty Incidents; Emergency Responders; Inservice Training; Disaster Planning; Video Games; Computer Simulation; Ethics; Cost-Benefit Analysis; Quality Improvement; Risk Management; Medical Errors; Learning

Sažetak

Uvod. Brzi razvoj multimedijalnih tehnologija u poslednjih 20 godina doveo je do nastanka novih načina učenja akademskih i profesionalnih veština primenom multimedijalnih tehnologija u formi softverskih proizvoda − *ozbiljnih kompjuterskih igara*. **Trodimenzionalni virtuelni svet**. Osnovu platforme ovih igara čine trodimenzionalni virtuelni svetovi koji se mogu opisati kao komunikacioni sistemi u kojima učesnici dele isti trodimenzionalni virtuelni prostor i mogu da se kreću, manipulišu predmetima i komuniciraju preko svojih grafičkih autoprezentacija − avatara. **Obuka u virtuelnim svetovima.** Argumenti za korišćenje kompjuterskih alata u procesu učenja su: laka pristupačnost, ponovljivost, niska cena, mogućnost korišćenja atraktivnih grafika i visok stepen prilagođavanja korisniku. Specifično dizajnirani avatari omogućavaju učenicima da se adaptiraju na svoje uloge u određenim situacijama, pogotovu onim koje su u realnom životu smatraju retkim, opasnim ili neetičnim. Treniranje velikih incidenata, koje uključuje i potrebu kreiranja okruženja za obuku, ne može se sprovesti u realnom svetu zbog visoke cene, neophodnosti angažovanja obimnih resursa i nemogućnosti istovremenog angažovanja potrebnog zdravstvenog osoblja. **Diskusija**. Nove tehnologije za sprovođenje treninga, virtuelni svetovi, omogućavaju: trening u bilo koje vreme kada to druge obaveze dozvoljavaju; istovremene simulacije na više nivoa, u više oblasti, u različitim okolnostima i sa desetinama jedinstvenih žrtava; ponavljanje scenarija i učenje na greškama; brze povratne informacije i razvoj netehničkih veština (efektivna komunikacija, liderstvo, upravljanje stresom, svest o situaciji) koje su kritične za redukciju grešaka u dinamičnim, visokorizičnim okruženjima. **Zaključak**. Virtuelni svetovi, uz potrebu za daljim istraživanjima i poboljšanjima, svakako predstavljaju, u domenu obuke zdravstvenih timova u reagovanju na javnozdravstvene krize, budućnost koja obećava.

Ključne reči: masovne katastrofe; zdravstveno osoblje; stručna obuka; rešavanje problema u katastrofama; video igre; kompjuterska simulacija; etičnost; isplativost; unapređenje kvaliteta; upravljanje rizikom; medicinske greške; učenje

Introduction

New ways of mastering academic and professional skills using multimedia technology in the form of software products and educational computer games have been developed in the last few years [1]. Although the idea of video games as an effective learning instrument is not a new one, modernization and financial

Corresponding Author: Dr Vojislav Stanojević, Medicinski centar Zaječar, 19000 Zaječar, Rasadnička bb, E-mail: svojkan@beotel.net

Abbreviations
3-D – three-dimensional
VED – virtual emergency department

pressure imposed on contemporary health systems urged the development of new methods of training, which are both time and cost-effective [2].

The rapid development of multimedia technology during the last twenty years has led to an increased worldwide use of computer games [1, 2]. Gaming consoles, computers, and mobile phones allow online advanced-technology gaming with people all over the world in the very comfort of your own home. Many of these games have been developed for educational purposes and are described as games with a useful purpose, which are not created for pleasure or serious games [2].

Three-dimensional virtual worlds

Along with the growing interest for serious games, the number of three-dimensional virtual worlds (3-D virtual worlds) has grown simultaneously; 3-D virtual worlds being the very basis of the gaming platform [3]. Based on the development of the internet, multimedia technologies and high power graphic cards which offer the possibility of creating a three-dimensional representation and realistic interactive environments [4, 5], virtual worlds can be described as communication systems in which participants share the same three-dimensional virtual space and can move freely, manipulate objects and communicate *via* their graphics representations, *avatars* [6, 7].

Through sensory information, three-dimensional virtual worlds offer the users a sense of real-time experience known as presence - presence in the virtual world, while actually sitting at their desk at home. This presence is experienced through *merging* while becoming aware of the environmental senses through a continuous stream of experience and stimuli, through co-presence (people see other avatars as representatives of real people), and real-time situations (the extent to which other objects, including avatars imitate reality) [8]. The expression avatar originates from Sanskrit and denotes a deity taking a human form [3]. These animated figures, avatars, are managed in such a manner so that they can run, walk, swim, go through closed doors, open drawers, etc. [3, 8]. Avatars interact with other avatars and communicate via text or voice messages using a headset and a microphone [8]. Thus, instead of being a passive observer of the image on the screen, the user becomes an active participant in a computer-created virtual world which enables him to learn, socialize and behave in a way similar to the behaviour in real-time and to practice new behaviours in realistic scenarios without the risk of making mistakes as in the real world [8].

Three-Dimensional Virtual Worlds in Medical Education and Training – Pro et Contra

One of the objectives of education and training of health professionals implies the creation of teams of practitioners with the knowledge and skills enabling them to work competently and in a safe environment [9]. Although the health care system emphasizes the team activity, given that team training is the most effective way of improving team performance, the efforts are generally focused more on individual training, probably because training sessions need to be coordinated with the individuals' commitments and with team members joining the team from different locations [10]. This supports the tradition of individual training in medicine, while patients remain the primary medium. However, all of the procedures to be performed on the patient are designed from the aspect of patient's care, and not from the educational one. The increased risk of complications, inability to repeat procedures and learning from mistakes, and the fact that the procedures can be learned only on an adequate patient, make the *living patients* poor instrument of training, requiring from medical personnel to learn their skills and exercise away from the patient's bed [2]. Arguments in favour of the use of computer tools in the learning process are: ease of accessibility, repeatability, low cost, possibility to use attractive graphics, a high level of adjustment to the user who accepts games as interesting and useful activities [1]. Compared to conventional teaching instruments (books, lectures, mentor's instructions), virtual worlds include more instruments which imply critical thinking, collaboration, and teamwork [2] as well as components of social learning (conversations and interactions) that represent the attributes of effective teaching [8]. Specifically designed avatars allow students to adapt to their roles in given situations, especially those considered to be rare, dangerous or unethical in real life [7]. The acquired understanding of clinical situations is applied in real-time and is more effective than simply memorized facts [2]. Gaming anonymity allows better progress, even for people with higher levels of inhibition (63% of participants are much more relaxed, and 56% more active than during traditional classroom teaching) [2, 7].

One of the concerns is the fact that people who have never played computer games may lack computer skills, which can create difficulties in learning and training processes, affecting the users' abilities to focus on the training objectives, because they are busy using computer tools, managing the screen and understanding the game [2, 7]. The increasing use of computers in everyday life makes this issue seem rather insignificant [2]. The objective problem for clients unaccustomed to install software components into their computer systems can be found in rapid technological changes of platforms that require frequent software updates. The solution to this problem lies in the fact that virtual environments should become part of standardized interface of web browsers, which would reduce the need for software updates [7].

Healthcare Team Emergency Response Training for Mass Casualty Incidents

After September 11th 2001, the expression *medical disaster emerged* and events including a large number of victims were placed highly on the list of public

health priorities. Even though these events are still considered as *the events of low probability* [11], disasters whether caused by natural events, infectious diseases, terrorism or technology [12] occur at a rate of one disaster a day [4]. During 2011, the world suffered 325 catastrophic events (175 natural and 150 caused by men) which forced nearly 15 million people to leave their homes [4]. Large-scale events have shown that disasters are almost inevitable and that health-care professionals must be ready to respond promptly and on time [13]. Some most disastrous events are the collapse of the World Trade Centre in 2001, Hurricane Katrina in 2005 - destroying 90 000 square miles of State Mississippi and flooding New Orleans thus causing 1,500 victims [14]; a fire in "Kiss" nightclub in Santa Maria, Brazil in 2013 with 234 victims, while almost 800 people requested medical assistance [15], and the terrorist attacks in Paris in 2015 with 150 victims. During the past decade, governments, public health organizations and hospitals were asked to develop action plans so as to react to disaster resulting in a large number of victims caused by different agents, wherein the component of training in all plans was inevitable. The Joint Commission on Accreditation of Healthcare Organization (JACH) included the United States Hospital Emergency Preparedness Program in the accreditation standards and demanded training implementation twice a year [16].

Although training of health personnel represents a critical component within the program for disaster preparedness, gaps in education and training [4] show that the existence of *the book knowledge* does not necessarily result in the transfer of this knowledge into practice [17]. There is no such training which can absolutely prepare clinicians to react in real incidents involving a large number of victims [13]; the current standard of training is limited by several factors, primarily by credible real-time situations which are very difficult to achieve [17]. Types of disasters and their locations can be very different and cannot be completely replicated. In real-time situations, the personnel must work in conditions of physical and emotional stress, in a potentially unstable and dangerous environment with many injured, disoriented and panicked people. Real-time environment and the scale of trauma are usually completely different from anything that the medical personnel experienced or practiced through training. This discrepancy between their knowledge and beliefs and the reality exposes practitioners to unstable conditions that can induce negative emotions (anxiety, anger, guilt) which have adverse effects on their performance, jeopardize the decision-making process and lead to degradation even of those skills that were routinely practiced [13, 18]. Mastering the knowledge and skills of affective control resulting in less vulnerability to external stressors cannot be a part of the given training [13]. This type of training is very expensive (USD 35,000-1,000,000), engaging various types of human resources (volunteers, moulage teams, security), materials, equipment and environment (e.g. closing an entire block to simulate a terrorist incident) [13, 18]. It is very difficult, almost impossible, to engage a large group of healthcare personnel, take them away from their daily duties [11, 17]. Due to high costs and other constraints, these exercises do not provide much opportunity for multiple test skills, and the preparation of post-action reports can take months, therefore participants usually receive only minimal feedback on the evaluation of exercises [17].

It has become clear that training to handle major accidents, including the need to create a learning environment, cannot be implemented in the real world [19]. This introduced the application of new technologies for training implementation, such as virtual worlds, which would enable the simulation of assessment and management of large numbers of victims of probable disasters on different levels and locations [20]. The efficient and practical model of gaming techniques of serious games, such as avatar model, provides a number of advantages within training in virtual worlds [11]: 1) training can be done at any time of day or night when the participants do not have to look after their patients; 2) participants do not have to be at the same location to manage avatars; 3) modifications can be made in different environments and conditions in order to simulate the complexity of real life situations; 4) scenarios can be reloaded (learning from mistakes); 5) the virtual environment allows simultaneous simulations on multiple locations or in multiple facilities and the presence of tens of unique victims in different conditions (crowd, dark, smoke, noise, damage to infrastructure, contamination, infection) which enable the participants to practice their individual and team skills under conditions highly similar to real-time ones; 6) all activities during training are automatically recorded and can be reviewed during the post-action period. Training in virtual worlds also enables the development of non-technical skills (effective communication, leadership, stress management, awareness of the situation) that are critical for reducing errors in dynamic high-risk environments [18]. Despite initial expenses put in to create and develop virtual environments (USD 20,000 to 100,000), virtual training is a financially more acceptable solution than *live* disaster exercises because these expenses are compensated for by a large number of users, applications and scenario reloading [13]. Virtual environment platforms include different components of persuasion, integrative information technologies designed to change users' attitudes and patterns of behaviour [10].

Virtual Worlds for Healthcare Teams Training

Systems for emergency response training for mass casualty incidents have a wide range of technical options, from personal computer software to fully immersive platforms of high credibility where students work with the help of three-dimensional googles or head-mounted displays [18]. For the purposes of this study, we will present some of the most common applications that apply to personal computers and software with broadband internet connection.

System for Triage Training and Incident Management

This computer-based system for disaster simulation in virtual reality, developed by E-Semble BV (Delft, Netherlands) and the Centre for Teaching and Research *Trauma*, Acute Care, and *Disaster Medicine* of the University of Linköping, Sweden, is used for training in fourteen European countries. The system introduces realistic scenes, with minimal programming effort, including any number of motor vehicles (buses, trains, planes, vehicles for emergency response), people (observers, victims, police officers, firefighters, and medical personnel), different sceneries, and disaster situations (fires, explosions, gas leaks, etc.). Using a control pad, the users move through the sceneries, examining each victim. By clicking on the victim, a descriptive palette in Excel appears on the screen with all information about the victim and their physiological status. Clinical information necessary for triage is obtained by clicking on the appropriate part of the body of the victim. Assessment of the victim's palpable pulse, for example, is done by clicking on the wrist victim; a "yes/no" answer is obtained from the victim's database. When the user has sufficient information to determine the triage category, he uses the control pad to select the red, yellow, or green category to mark the victim. All activities of the participants are automatically entered in the Excel table, which allows post-action analysis of the length of each step in the selection process and the sequence of the examination of clinical parameters [4].

Triage Trainer

The scenario predicts a bomb blast in a crowded city street, the scenery is showing the destruction of infrastructure with numerous victims around (3-10 victims). The user applies the cursor to reach the victim, and checks on the medical status by clicking on the victim. After checking the victim, using the priority icon, the user determines the victim's priority for the triage process. After completing triage in post-action phase, the user checks the precision within the triaging process, where he points to the correct steps in the assessment of each victim but also points to any mistakes made during the process [19].

Virtual Emergency Department I

Based on the Atmosphere platform, beta version of the software by Adobe Systems Inc, Virtual Emergency Department I (VED I) represents a virtual emergency department including six injured patients [11]. Ten clinical scenarios introduce the patients with injuries such as rupture of the spleen, thoracic injuries, fractures of extremities, etc. The trauma team must evaluate the status of each patient and begin appropriate treatment. The vital signs of patients (heart rate, blood pressure, respiratory rate, and oxygen saturation) are designed to demonstrate a favourable response if the appropriate clinical activities are selected. If the clinical priorities are incorrect, vital signs will worsen in ten minutes, causing *death* of the virtual patient. In order to get an improved sense of engagement inside the trauma team, the virtual patients' conditions are changed 50% faster than in real-time. Members of the trauma team enter the VED by clicking on a hyperlink on a standard HTML web page. Using the mouse or the direction keys on the keyboard, they move their avatars to perform various diagnostic and therapeutic activities (over 30 of these activities are programmed) by choosing an option from the Control menu. In order to complete certain diagnostic activities, the participants must position their avatars minding the patients correctly. Each participant communicates with the patients and other team members by using a headset. Avatar's activities are visible to other team members, but the results of the activities are shown only to the team member who had performed those activities. Thus, the team members must share information about their activities verbally in order to be a functional team because effective communication among team members is of critical importance in real life [21].

Virtual Emergency Department II

Virtual Emergency Department II (VED II) was developed using the Olive platform by gaming experts of Forterra Systems Inc. and represents a replica of the emergency room (ER) department at the Stanford University Medical Centre. The platform includes twenty different scenarios in which the patients' avatars are presenting with signs and symptoms of poisoning due to exposure to nerve gas (sarin) in a train and to trauma caused by the explosion of a *dirty bomb* in a local bank [11]. The Olive platform enables participants to conduct triage outside the hospital, while the other team simultaneously conducts triage inside the hospital. The patients are represented with ten avatars of different ages and sexes with different comorbidities and injuries. The vital parameters of patients are programmed to deteriorate at various speeds, depending on the severity of injuries. Without proper treatment, most patients subject to injury thirty minutes after arriving at the hospital [20].

Discussion

Virtual worlds have become a part of everyday life of a great number of people who actually rely on them in different segments, so that virtual and real-time activities progressively intertwine. Virtual worlds are not only 3-D multiplayer games. The seriousness of these *serious games* provide a true experience of real presence in virtual environments, social networking in real-time and a unique form of social network interaction, including creative collaboration.

The emergence of platform technology for virtual reality applied in response training for mass casualty incidents including a large number of victims offers significant advantages over other traditional training standards. The immersive and participatory nature of training in virtual worlds gives a unique realistic quality of training, which is generally not present in other modalities of teaching and retains a large cost advantage over different forms of training in real life [18].

The educational usefulness of serious games within response training for mass casualty incidents can certainly be and should be discussed. Further research is necessary for their validation in relation to already existing methods of training in this field [2]. Therefore, it is necessary to determine whether there is an improvement in triage, intervention and efficiency scores by using serious games and how these scores correlate with the scores achieved by traditional models of training [2, 17]. The triage training for major incidents, where teamwork is most important, showed a great performance improvement introducing a combined system of conventional methods of training and serious games [2].

Conclusion

Managing mass casualty incidents which include a large number of victims is only possible with adequate training, difficult to implement in real-time. Virtual worlds, which should be the subject of further research and improvements, in the field of hospital emergency response training for mass casualty incidents, certainly have a promising future.

References

1. Girard C, Ecalle J, Magnan A. Serious games as new educational tools: how effective are they? A meta-analysis of recent studies. J Comput Assist Learn. 2013;29:207-19.

2. Kitchen GB, Humpreys J. Serious games in medical education. Do they have a role in anaesthetic training. Trends in anaesthesia & critical care. 2014;4:63-6.

3. Hansen MM. Versatile, immersive, creative and dynamic virtual 3-D healthcare learning environments: A review of the literature. J Med Internet Res. 2008;10(3):e26.

4. Farra SL, Miller ET. Integrative review: virtual disaster training. J Nurs Educ Pract. 2103;3(3):94-101.

5. Cone DC, Serra J, Kurland L. Comparison of the SALT and Smart triage systems using a virtual reality simulator with paramedic students. Eur J Emerg Med. 2011;18(6):314-21.

6. Ghanbarzadeh R, Ghapanchi AH, Blumenstein M, Talaei-Khoei A. A decade of research on the use of three-dimensional virtual worlds in health care: A systematic literature review. J Med Internet Res. 2014;16(2):e47.

7. Surakka T. Different virtual environments – something for everyone. Available at: http://www.inter-disciplinary.net/wp-content/uploads/2012/02/teemuepaper.pdf

8. Johnson CM, Vorderstrasse A, Shaw R. Virtual Worlds in Health Care Higher Education. Journal of virtual worlds research. 2009;2(2):3-12.

9. Blakely G, Skirton H, Cooper S, Allum P, Nelmes P. Educational gaming in the health sciences: systematic review. J Adv Nurs. 2009;65(2):259-69.

10. Khanal P, Vankipuram A, Ashby A, Vankipuram M, Gupta A, Drumm-Gurnee D, et al. Collaborative virtual reality based advanced cardiac life support training simulator using virtual reality principles. J Biomed Inform. 2014;51:49-59.

11. Koenig KL. Editorial comments-training healthcare personnel for mass casualty incidents in a virtual emergency department: VED II. Prehosp Disaster Med. 2010;25(5):433-4.

12. Wong D. Managing mass casualty events is just the application of normal activity on a grander scale for the emergency health services. Or is it? Journal of Emergency Primary Health Care. 2011; 9(1): Article 990448.

13. Andreatta PB, Maslowski E, Petty S, ShimW, Marsh M, Hall T et al. Virtual reality triage training provides a viable solution for disaster-preparedness. Acad Emerg Med. 2010;17:870-6.

14. Becerra-Fernandez I, Madey G, Prietula M, Rodriguez D. Project ENSAYO: a virtual emergency operations center for disaster management research, training and discovery. Proceedings of the Second International Conference on Internet Monitoring and Protection; 2007 Jul 1-6; Silicon Walley, USA. Available at: http://www3.nd.edu/~dddas/Papers/madeyg-ensayo.pdf.

15. Dal Ponte SD, Dornelles CFD, Arquilla B, Bloem C, Roblin P. Mass-casualty response to the Kiss Nightclub in Santa Maria, Brazil. Prehosp Disaster Med. 2014;30(1):93-6.

16. LeRoy H, Youngblood P, Hartner P, Kusumoto L, Parvati D. Training healthcare personnel for mass-casualty incidents in a virtual emergency department: VED II. Prehosp Disaster Med. 2010;25(5):424-32.

17. Wilkerson W, Avstreih D, Gruppen L, Beier K-P, Woolliscroft J. Using immersive simulation for training first responders for mass casualty incidents. Acad Emerg Med. 2008;15:1152-9.

18. Hsu EB, Li Y, Bayram YD, Levinson D, Yang S, Monahan C. State of Virtual Reality Based Disaster Preparedness and Response Training. PLoS Curr. 2013; 5. Available at: http://currents.plos.org/disasters/article/state-of-virtual-reality-vr-based-disaster-preparedness-and-response-training/.

19. Knight JF, Carley S, Tregunna B, Jarvis S, Smithies R, De Freitas S. et al. Serious gaming technology in major incident triage training: A pragmatic controlled trial. Resuscitation. 2010; 1175-9.

20. LeRoy H, Youngblood P, Hartner P, Kusumoto L, Parvati D. Training healthcare personnel for mass-casualty incidents in a virtual emergency department: VED II. Prehosp Disaster Med. 2010;25(5):424-32.

21. Youngblood P, Harter PM, Srivastava S, Moffett S, Heinrichs WL, Dev P. Design, development, and evaluation of an online virtual emergency department for training trauma teams. Simul Healthc. 2008;3(3):146-53.

Rad je primljen 9. II 2016.
Recenziran 9. III 2016.
Prihvaćen za štampu 11. III 2016.
BIBLID.0025-8105:(2016):LXIX:7-8:255-259.

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

Korisnici časopisa treba da se registruju na adresi:
http://aseestant.ceon.rs/index.php/medpreg/user/register

Prijava rada treba da se učini na adresi:
http://aseestant.ceon.rs/index.php/medpreg/

U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

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

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

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

Manuscript submission should be made on the web address:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

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

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

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

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

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

3. The text of the paper.

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

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

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

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

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

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

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *A computer file*

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

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

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

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

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

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

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

– 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 color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.